Abstracts of the 12th International Symposium on Neural Regeneration

ORAL PRESENTATIONS

O-1 Men On Wheels, Men On Fire: Sodium Channels As Molecular Targets In Disorders Of The Spinal Cord

S.G. Waxman

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Voltage-gated sodium channels are widely expressed within neurons and play pivotal roles in neuronal signaling. Less has been known about the roles of sodium channels in disorders of the spinal cord and brain. Neurophysiology classically referred to "the" sodium channel as if it were a singular entity. Recent research has taught us that sodium channels are more complex than previously appreciated, and has shown us that sodium channels play multiple roles in neuronal pathophysiology.

In this lecture I will review the following advances: 1) we now understand that ten different genes encode ten different isoforms of sodium channels, with different kinetic and voltage-dependent properties. 2) changes in expression of sodium channels play an adaptive role in some demyelinated axons, and provide a molecular substrate for clinical remissions in disorders such as MS, suggesting that it may be possible to use molecular methods to induce remissions in MS and possibly in non-penetrating SCI, where a subset of axons at the level of the lesion can survive but fail to conduct impulses due to demyelination. 3) other changes in expression of sodium channels are maladaptive, an example being neuropathic pain, where dysregulated expression of sodium channels can lead to abnormal high-frequency firing of neurons along the pain-signaling pathway in the absence of external painful stimuli. 4) we now understand, for the first time, a human hereditary pain disorder (erythromelalgia, the "man on fire syndrome") which is due to a gain-of-function mutation in a sodium channel that makes nociceptive DRG neurons hyperexcitable, providing a model in humans of chronic pain due to sodium channel dysfunction. 5) we have begun to understand the role of sodium channels in axonal degeneration in disorders such as MS; this suggests that it may be possible to protect axons so that they do not degenerate in these disorder by targeting the offending sodium channels. The path to clinical trials has had some interesting turns, however, and I will conclude by discussing the complexity of the path from laboratory bench to clinic.

References:

Waxman, S.G., Channel, neuronal, and clinical function in sodium channelopathies: From genotype to phenotype. Nature Neuroscience, 10:405-410, 2007.

Waxman, S.G. Axonal conduction and injury in multiple sclerosis: the role of sodium channels.Nature Rev. Neurosci., 5: 932-942 (2006).

Waxman. S.G., Hains, B.C. Fire and phantoms after spinal cord injury: sodium channels and central pain. Trends in Neurosciences 29: 207-215. 2006

Waxman, S.G., Dib-Hajj, S.D.Erythermalgia: molecular basis for an inherited pain syndrome, Trends in Molecular Medicine, 11 (12): 555-562, 2005

O-2 **Modeling Axon Guidance During Reinnervation**

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Overcoming barriers to reinnervation following peripheral nerve, spinal cord or traumatic brain injury is a major research challenge. New model systems are needed to test ways of stimulating neurons to overcome barriers to reinnervation while retaining guidance. To reach target areas, neurons send axons long distances through a complex and dynamic environment. Regenerating axons are usually hindered by an environment that is inhibitory to growth and lacking the appropriate guidance factors. Axons are guided by molecular cues that interact with cell surface receptors. Most of the receptors are concentrated in the growth cone, a dynamic structure at the tip of a growing axon. Many potential guidance cues have been identified; however, their relative strengths, the signaling derived from receptor activation and how it is integrated to affect growth cone motility are not well understood.

Using novel *in vitro* technology for substrate patterning, we are testing the response of growth cones to specific sets of cues. We are also testing the role of myosin II in regulating the growth cone response. With this approach we are able to rank guidance cues and determine whether the hierarchical responses converge to the same effector mechanism, namely myosin II-dependent turning. Our results indicate the signaling pathways for laminin and nerve growth factor are separate and competitive. Using this information we are creating surfaces with cues in specific patterns to control branching and turning. Ultimately our *in vitro* model system will allow us to determine substrate compositions that stimulate and guide outgrowth, overcoming conditions that are normally inhibitory such as occurs during reinnervation. Supported by NINDS NS26150.

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O-4 **The Long And Winding Road: Axon Regeneration Within The Optic Nerve.**

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The injured optic nerve is an extremely hostile environment for axon regeneration. Unlike the spinal cord in which most neurons atrophy after injury, but can remain in “suspended animation” for years, retinal ganglion cells (RGCs) die rapidly (within days) after axotomy. Once they die, RGCs disappear from the retina and optic nerve. Many investigators, including us, have tried to rescue RGCs and promote regeneration by injecting neurotrophic factors into the eye with limited success. However, we recently demonstrated that fibroblast growth factor-2 (FGF-2), unlike other neurotrophic factors, promotes robust RGC axon regeneration after optic nerve lesion. FGF-2 gene transfer, using recombinant adeno-associated virus (AAV) technology, resulted in a 10-fold increase in the number of regenerating RGC fibres with respect to control nerves. Furthermore, we recently demonstrated that FGF-2 strongly activates retinal Erk1/2 *in vivo*, and that pharmacological inhibition of Erk1/2 blocked FGF-2-induced regeneration. Thus, Erk1/2 is a required intermediary for FGF-2-induced RGC axon regeneration *in vivo*. We are also interested in the role of receptor protein tyrosine phosphatases (RPTP), which regulate the activity of protein tyrosine kinases, in axon growth. We recently examined RGC axon regeneration in RPTPσ knockout mice. A striking increase in the number of regenerating axons in injured optic nerves from adult RPTPσ (-/-) compared to wild-type littermate controls was found. The retinas and optic nerves of RPTPσ deficient mice showed no histological defects and the time-course of RGC death after lesion was identical between knockout and wild-type animals. Therefore, enhanced axon regeneration in the absence of RPTPσ could not be attributed to developmental defects or increased

survival. Our study provides the first demonstration that RPTPσ inhibits axon regeneration in the injured visual system, and identifies a novel class of growth inhibitory molecules in the CNS.

O-5 **Imaging Regeneration In Vivo**

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Many of the controversies surrounding experiments in regeneration could be resolved by directly imaging the process, in vivo, in the spinal cord. We have done this by taking advantage of the transparency of larval zebrafish. Our work shows that direct application of membrane permeable cyclic AMP to the somata of reticulospinal neurons in hindbrain can induce sprouting and regeneration across the lesioned spinal cord. This regeneration is associated with the recovery of activity patterns distal to the lesion site, as assessed by calcium imaging of activity of the same neurons before and after lesions. The behavior produced by the lesioned neurons recovers as well, indicating that cyclic AMP alone can go a long way toward solving the problem of regeneration in this model. The techniques are available to apply similar in vivo imaging in mice and this should allow for a more rapid and conclusive assessment of the prospects that particular drugs or combinations of drugs might solve the problem posed by spinal injury.

O-6 **Cell Adhesion Molecule And Nervous System Regeneration**

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Cell adhesion molecules play important roles not only during ontogenetic development of the nervous system, but also in the adult during regeneration after acute and chronic lesions, and in synaptic plasticity underlying learning and memory (for recent reviews see Loers and Schachner, 2007; Maness and Schachner, 2007). Among these molecules are transmembrane glycoproteins of the immunoglobulin superfamily, such as L1, the close homolog of L1 CHL1 and the neural cell adhesion molecule NCAM. Extracellular matrix molecules have been shown to be equally important in their functional impact both as promoters and inhibitors of regeneration. The importance of glycans for regeneration is increasingly being recognized. My presentation will present evidence on the functional properties of beneficial and adverse impacts of both immunoglobulin superfamily adhesion molecules and extracellular matrix glycoproteins on regeneration after spinal cord injury in the adult mouse. Furthermore, experiments will be presented showing that overexpression of the immunoglobulin superfamily adhesion molecule L1 by embryonic stem cells or application of adeno-associated virus encoding for L1 to the lesioned spinal cord and in other acute lesion paradigms of the central nervous system promote functional recovery. Surprisingly, CHL1 is adversive to regeneration. Also surprisingly, the extracellular matrix glycoprotein tenascin-C is conducive to regeneration, whereas its close homolog, the extracellular matrix glycoprotein tenascin-R reduces regeneration. Adhesion molecule-associated carbohydrates, such as the human natural killer cell antigen 1 (HNK-1) and the neural cell adhesion molecule NCAM associated polysialic acid enhance regeneration in the central and peripheral nervous system of adult mice and, for the peripheral nervous system also of monkeys, when applied as glycomimetic peptides. It appears that combinatorial approaches in targeting will be important for therapy.

References:
Loers G, Schachner M (2007) Recognition molecules and neural repair. J Neurochem 101:865-882.

Maness PF, Schachner M (2007) Neural recognition molecules of the immunoglobulin superfamily: signaling transducers of axon guidance and neuronal migration. Nat Neurosci 10:19-26.

O-7 **Neurodegeneration And Regeneration In The Nervous System**

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We have explored several stem cell-based therapies for refractory neurologic diseases including multiple sclerosis (MS), transverse myelitis (TM) and spinal muscular atrophy (SMA). In this talk, I will discuss the status of three of these projects. In the first, high-dose cyclophosphamide (Revimmune) is explored as a one-time front-loaded therapy to induce long term remission in patients with MS. Revimmune works by temporarily eliminating maturing and mature immune cells while sparing bone marrow stem cells, thus allowing reconstitution of a naïve immune system without transplantation. As part of a clinical protocol for aggressive MS, 10 patients have been treated with Revimmune and then no subsequent immunomodulatory therapy for up to 24 months. There was a 90% reduction in gadolinium enhancement by 18 months, a 95% reduction in exacerbation frequency and a 45% reduction in disability.

Preclinical studies in my laboratory focus on the use of glial restricted precursors (GRPs) as potential therapeutic agents for CNS demyelinating disorders. Following completion of the preclinical studies, we hope to initiate a single center, open label pilot study with dose escalation to obtain preliminary data on the safety and tolerability of GRP cells in patients with disability from TM.
We recently defined the ability of embryonic stem cell-derived motor neurons to functionally replace those destroyed in paralyzed adult rats. This is the first report, to our knowledge, of the anatomical and functional replacement of a motor neuron circuit within the adult, mammalian host. We are currently carrying out large mammal studies to generate the necessary preclinical data and initiate a clinical trial of ES cell-derived motor neurons to reconstitute motor neurons damaged in infants with the fatal motor neuron disorder SMA.

O-8 **Diffusion Tensor Mri (Dti) And The Evaluation Of Axonal Integrity Following Spinal Cord Injury**

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Treatment options are limited for spinal cord injury, and methylprednisolone, the only treatment shown to be effective in ameliorating spinal cord injury, has been aggressively questioned over the past few years. Despite this setback in the clinical arena, basic science research has identified many promising treatment options; however, barriers to potential clinical translation remain. Determination of treatment efficacy in the experimental setting and in the clinical setting is often difficult. In the experimental setting, large numbers of animals are often required to provide histologic specimens at numerous time points, and the application of behavioral gains to the clinical setting is not always clear. In the clinical setting, early identification of patient populations for treatment delivery is important as optimal therapy may need to be delivered in the first few hours or days, yet neurological examination may take up to a week or longer before an accurate prognosis is obtained. Additionally, histologic data may not be available in the clinical setting and behavioral recovery may take years. In order to address these concerns, advanced MRI techniques have been identified by basic and clinical researchers as potential objective and quantifiable outcome measures that can serve as a bridge between the lab and the clinic. Diffusion tensor MRI (DTI) appears to be one of the most promising non-invasive outcome measures of spinal cord injury as it can be performed both in the lab and in the clinic. DTI evaluates the diffusion of water molecules, and, in a highly ordered structure such as spinal cord white matter, these diffusion parameters have been shown to correlate with quantitative axon morphologic parameters, such as axonal density and integrity. In this lecture, the basics of this imaging technique will be discussed, followed by a review on current research regarding DTI and spinal cord injury.

O-9 **The Resolution Of Complex Spinal Cord And Brain Structures In Vivo And Ex Vivo Using High Angular Resolution Diffusion Magnetic Resonance Imaging.**

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Recent advances in magnetic resonance imaging (MRI) have provided a method for visualizing the fibrous structure of the nervous system, e.g. white matter in the central nervous system. The method is based on the measurement of translational water diffusion throughout the tissue. Because the measured phenomena results from the thermally driven process of diffusion, the method can be used to visualize diffusion in living or excised tissue. Diffusion-sensitive MR images can be used in a straightforward way to infer the fibrous structure of tissue by modeling the diffusion process with a rank-2 tensor representation (the so called diffusion tensor image, DTI) of the three-dimensional translational displacement of the water in the fibrous tissue. In this talk, the use of DTI for the study of tissue structure will be illustrated with examples in the brain and spinal cord. However, DTI is inadequate to represent more complex tissue structures, such as crossing fiber regions. This is because, in each image voxel, only a single average fiber can be represented by a rank-2 tensor. Others have extended the diffusion measurement process using high angular resolution diffusion imaging to provide increased discrimination of the directionality of diffusion. Our current work as been directed at developing ways to model diffusion in complex tissue at higher resolution in order to resolve crossing fibers in white matter regions and to begin exploring the resolution of structures at the boundaries between white and gray matter and even into gray regions. Most of our efforts have been focused on experimental animal models of spinal cord injury and of epilepsy following brain injury. More recently, we have begun to translate our work with animal models to image humans in a clinical setting. Examples of this recent work will be presented in this talk.

O-10 **Clinical Applications Of Magnetic Resonance Imaging In Human Spinal Cord Injury**

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Magnetic Resonance Imaging (MRI) has revolutionized the diagnosis of diseases of the spine and spinal cord. In the setting of spinal cord injury (SCI), MRI has become an essential diagnostic tool in the initial period following injury and for long-term assessment when late neurologic deterioration occurs. MRI is the only non-invasive imaging tool that depicts the structure of the spinal cord. Since it was first applied in the setting of SCI, numerous experimental and clinical investigations have shown a direct correlation between the appearance of the damaged spinal cord on MRI and the degree of functional deficit at the time of injury and the capacity for neurologic recovery. The ultimate clinical value of MRI in SCI may be realized in the selection and monitoring of patients for novel forms of therapeutic intervention.

There are three standard MR imaging features that characterize SCI: spinal cord hemorrhage, spinal cord edema and spinal cord swelling. A typical SCI on MRI is spindle-shaped and characterized by a central focus of hemorrhage and a peripheral margin of edema that may span a variable length of the spinal cord. Edema appears brighter (hyperintense) relative to normal spinal cord parenchyma on T2-weighted images whereas subacute hemorrhage appears dark (hypointense). In some instances, no central focus of hemorrhage will be identifiable on MRI, in which case, only edema is present. The damaged region of spinal cord is usually larger in caliber (swollen) than the normal adjacent parenchyma. The MR features are often described in terms of presence/absence, location and extent. Moreover, these changes evolve in the acute period showing predictable increase in size in the first seventy-two hours.

Frank intramedullary hemorrhage is generally equated with a severe neurologic deficit and a poor prognosis. Cord edema alone is associated with mild to moderate initial neurologic deficits which may

have a better prognosis. Lesion length is proportional to neurologic deficit and inversely proportional to neurologic recovery. The anatomic location of the SCI lesion epicenter/hemorrhage corresponds to the neurologic level of injury. There is additional substantial evidence that suggests that the MRI changes of SCI offer prognostic information regarding neurologic recovery. Poor recovery from SCI is associated with severe cord compression, cord swelling, and abnormal signal on T1-weighted and T2-weighted images. Moreover, patients with persistent signal changes in the spinal cord on follow-up MRI examinations demonstrate little or no clinical improvement in ASIA grade whereas prognosis is improved for patients who demonstrated resolution of signal abnormalities.

O-11 **The Influences Of Diet And Exercise On Plasticity And Repair**

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Emerging evidence indicates that in addition to their roles in our everyday existence, exercise and other lifestyle modalities are crucial determinants of the repair capacity of the CNS after insults. Exercise regimens as short as 3 days can promote changes in synaptic plasticity while long-term training can provide sustained neural protection against insults. The timing for the application of exercise after brain and spinal cord injury seems crucial for optimal functional recovery. Exercise is part of the spectrum of experiences that we encounter in daily leaving; therefore, its action is likely influenced by other experiences. For example, new research indicates that dietary factors can also affect brain plasticity and function under homeostatic conditions, and modulate the repair capacity in the injured CNS. In particular, the docosahexaenoic acid (DHA) is one of the major omega-3 polyunsaturated fatty acids in the brain, and has been implicated in normal neurological development, maintenance of learning and memory, and neuronal plasticity. A diet enriched in DHA protects neurons from neurological damage and helps the brain to reduce the consequences of brain trauma. Our new research indicates that the effects of a DHA diet can synergize with those of exercise to boost recovery events after brain trauma. These findings also show that the effects of the DHA diet on the CNS share common molecular mechanisms with those employed by exercise, involving neurotrophins. In particular, the action of BDNF on circuit remodeling, synaptic facilitation and behavior are influenced by diet and exercise. Furthermore, exciting new research indicates that in addition to their effects on synaptic plasticity and cognitive ability, diet and exercise reduce the growth inhibitory capacity of the injured CNS. In particular, a concurrent regimen of a DHA enriched diet and exercise reduces the elevation of MAG and NogoA after injury, in a mechanism involving BDNF. The new understanding about the role of experience on functional restoration following insults to the brain and spinal cord has introduced the possibility to manipulate these factors to enhance the outcome of recovery (supported by NIH awards NS50465 and NS45804).

O-12 **The Utility Of Adjuvant Therapies To Enhance Cortical Plasticity And Recovery From Stroke**

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Recovery from brain injury can be thought of as a relearning process whereby spared neural tissue is retrained to compensate for lost or impaired functions. The neural mechanisms underlying recovery appear to involve reorganization of remaining neural circuitry through changes in synaptic connectivity within residual neural tissue. Therefore, manipulations that promote synaptic plasticity may enhance experience-dependent recovery of function after brain injury. We have developed a rodent model of cortical ischemia to test the viability of such treatments. The model involves training rats to criterion on a skilled forelimb reaching task prior to inducing focal ischemic infarcts via either electrocoagulation or chemical vasoconstriction of the middle cerebral artery. The animals are then given daily motor rehabilitation on the same skilled reaching task. Intracortical microstimulation is used to assess the topography of movement representations within residual cortex. Using this model we show

that direct electrical stimulation of motor cortex significantly improves performance on a skilled reaching task. The enhanced recovery is also associated with significantly greater motor map expansion and reorganization in residual cortex. The same model demonstrates that stochastic electrical stimulation of peripheral sensory afferents also induces enhanced motor recovery and motor map reorganization. Finally, administration of two different type IV phosphodiesterase inhibitors known to upregulate the cAMP/CREB pathway significantly enhanced motor recovery and motor map reorganization. The relationship between the amount of motor recovery and motor map reorganization in all three treatment interventions is also not linear. The results suggest that there may be a critical amount of cortical reorganization induced by adjuvant treatments that is required before motor recovery is significantly increased above that observed with standard rehabilitation.

O-13 **Long-Distance Axonal Sprouting After Injury To Cerebral Cortex Of Adult Mammals**

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After cortical injury, one mode by which functional remodeling of spared cortical structures could occur is through rewiring of its anatomical connections. Axonal sprouting immediately adjacent to a focal injury is well accepted, but the extension of this phenomenon to distant but interconnected areas has rarely been demonstrated in adult mammals. Large-scale corticocortical sprouting of axons in the adult CNS after damage is still a matter of debate. However, it appears that a cortical injury creates a particularly favorable environment for sprouting to occur in the remaining, intact cortex, since genes involved in growth promotion are known to be upregulated for considerable time after injury. Recent evidence now supports the notion that after focal cortical injury in adult rodents and primates, large-scale, long-distance alterations in axonal trajectories and termination patterns occur in the weeks to months following the injury. Challenging questions have now emerged regarding the underlying mechanisms of post-injury axonal sprouting and guidance, as well as the functional significance of altered cortical connection patterns.

O-14 **Can Locomotion Be Controlled By Peripheral Afferents After A Complete Spinal Cord Injury?**

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There are two important properties of the spinal cord that provide the possibility of using activity-dependent rehabilitative strategies to regain the ability to stand and to step following spinal cord injury. First, the spinal cord can receive and interpret the sensory information from the lower limbs that is associated with weight-bearing and stepping. Therefore, when the body is placed in a weight-bearing posture, the spinal cord will respond by generating the appropriate activation signals necessary to execute successful standing and stepping. In essence, the spinal cord itself is capable of generating the appropriate activation patterns to generate standing and stepping. The second important property to be discussed will be the ability to improve the functionality of these spinal sensory-motor circuits by practicing a specific motor task. Evidence will be presented which demonstrates the ability of rats , cats and humans to learn to stand and to step following a complete mid- thoracic spinal cord transaction and some features of the training paradigm that seem to be important in promoting learning. Evidence will also be presented which demonstrates the importance of modulating excitatory and inhibitory neurotransmitter systems within the spinal cord (both pharmacologically and by training) when combined with epidural stimulation in controlling the ability to regain standing and stepping capabilities after a severe and even complete spinal cord injury. In considering the potential for recovery and the mechanisms responsible for the recovery of postural and locomotor function following a spinal cord injury, there is a fundamental difference between a “complete” and an “incomplete” injury. These

mechanisms will be discussed relative to the potential of several intervention strategies to achieve regeneration and reconnectivity across a spinal lesion.

O-15 **Recovery From Stroke**

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Patients show spontaneous behavioral recovery during the weeks that follow a stroke, though this is generally incomplete. Observations from animal studies have characterized the cellular and molecular events that contribute to this behavioral recovery, and include angiogenesis, synaptogenesis, and qualitative/quantitative changes in neurons and glia. A number of studies have described exogenous interventions that target these events and improve outcome. These findings are now entering human studies and defining a relatively new approach to reducing disability after stroke: restorative therapies, which aim to improve outcome not by salvaging tissue but instead by enhancing repair of surviving elements. This approach attains particular importance given the difficulty of accessing many stroke patients acutely. A range of approaches is under investigation, including small molecules, growth factors, cell-based therapies, electromagnetic stimulation, neuroprosthetics, motor imagery, intensive training, and robotic devices. Human brain mapping provides insights into brain repair, at times when behavioral exam and anatomical imaging are silent. Brain mapping studies might help optimize prescription of restorative therapies, for example, by identifying patients most likely to respond, guiding features of therapy such as dose or duration, or serving as a surrogate marker. New insights into the genetics of brain repair might also be useful for optimally translating preclinical findings into effective human therapeutic strategies.

O-16 **Mitochondrial Etiology Of Aging And Age-Related Neurodegenerative Disease**

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The human cell is assembled from two different organisms: the nucleo-cytosol organism which specializes in cellular and tissue structure and whose genes are Mendelian and the mitochondrial organism which specializes in energy and whose genes are maternal and stochastic. Inherited pathogenic mitochondrial DNA (mtDNA) mutations have been linked to a wide range of metabolic and degenerative diseases. Somatic mtDNA mutations accumulate with age in a broad spectrum of organisms, introduction of catalase into the mouse mitochondrial matrix reduces the mtDNA somatic mutation rate and extends life span, increasing *Drosophila* cAMP levels reduces mitochondrial reactive oxygen species (ROS) and extends life span, and treating short-lived *Drosophila* mutants with mitochondrially-targeted antioxidants can restore the life span. Ancient adaptive mtDNA polymorphisms have been associated with altered risk for metabolic and neurodegenerative diseases, such as metabolic syndrome and Parkinson disease, and somatic mtDNA mutations are elevated in the brains of Alzheimer Disease patients and Down Syndrome patients with dementia. Finally, both germline and somatic mtDNA mutations are associated with various cancers including prostate cancer. Therefore, diseases which appear “complex” when viewed exclusively from the nucleo-cytosol perspective might be more readily understood if the contribution of the mitochondrial organism were also considered.

O-17 **Differentiation Of High Purity Human Cell Populations From Human Embryonic Stem Cells**

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The development of research and clinical programs would benefit from a source of high purity human cell populations that are destroyed during the course of a particular injury or disease. Interest in human embryonic stem cells (hESCs) arises from their ability to provide an apparently unlimited cell supply for transplantation, and from the hope that they can be directed to desirable phenotypes in high purity. My research group has recently demonstrated that hESCs can be restricted in their differentiation potential to yield high purity cultures, demonstrating for the first time that hESCs can be manipulated to yield a high purity neural subpopulation. Here, we present those data, and other preliminary data that indicates that hESCs can be restricted in their differentiation potential to high purity motor neuron progenitor cultures. These data define a protocol for the generation of functional human motor neuron progenitors in high purity, to provide a biological tool to investigate human motor neuron development and a clinically relevant cell population for therapeutic development. The principles used in these high purity derivations will be discussed, as well as the application of the derivates to animal models of human injury and disease.

O-18 **NEURAL SUBTYPE SPECIFICATION FROM HUMAN ESCs**

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Human embryonic stem cells (hESCs), initiated from the inner cell mass of an early embryo, offer an unprecedented tool for unveiling normal and abnormal human development as well as developing medical therapeutics. We have established a chemically defined neural differentiation system that recapitulates most of the key events that occur during early human embryo development, such as formation of neural tube-like rosettes and appearance of region-specific progenitors at the correct time. With this model system we have discovered that the neuroectodermal differentiation from hESCs undergoes two morphologically and molecularly distinguishable stages, which we term the primitive and the definitive neuroectodermal cells. The uniform expression of anterior transcription factors in the primitive neuroepithelial cells, as opposed to the predominant mid/hind brain neuroepithelial differentiation from mouse ESCs, reflects the need of building the large forebrain in primates. Our differentiation model hence sets a foundation for revealing genetic and epigenetic programs underlying the making of our brain.

The two-step neuroectodermal differentiation model lends a conceptual framework for correctly patterning the naïve neuroepithelial cells, thus leading to successful differentiation of forebrain glutaminergic and GABAergic neurons, midbrain dopamine neurons, spinal cholinergic motor neurons, and myelinating oligodendrocytes. Systematic electrophysiological and transplantation analyses indicate that neurons produced in a Petri dish function like authentic neurons, respond to the brain environment for maturation, and correct functional deficit in neurological animal models. The in vitro produced oligodendrocytes can produce myelin sheaths in the brain of myelin deficient animals. Thus, the in vitro generated neural cells may be useful for potential future cell therapy. (Supported by NIH [NS045926, NS046587, NS061243], ALS Association, Michael J. Fox Foundation, and National MS Society).

O-19 **Human Es Cells In Development And Neural Repair**

L. Studer

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Human ES cells are characterized by their extensive self-renewal potential and pluripotency. However, it has remained challenging to direct differentiation of human ES cells towards pure populations of specialized cell types for cell repair or disease modeling. Here we will present data on a new technique based on BAC transgenesis to genetically mark and purify specific neural derivatives from mouse and human ES cells. Other techniques critical in the human ES cell field include the use of highly purified cell populations in high-throughput screening (HTS) assays to systematically identity compounds

that affect stem cell fates. We will provide an example on how human ES cells can be adapted to an HTS platform and how such assays could be developed in the future for drug discovery.

The use of specific neuronal derivatives from human ES cells requires an understanding of the signals that control regional specification in neural development and the identification of the developmental stages capable of responding to appropriate developmental signals. We have identified and characterized an early neural stem cell stage termed rosette neural stem cells (R-NSCs) derived from human or mouse ES cells that is susceptible to developmental patterning cues. Our data suggest that R-NSCs have specific signaling requirements and genetic make up compared with developmentally later more restricted NSC stages.

Applications based on the isolation, patterning and differentiation of R-NSCs include the derivation of midbrain dopamine neurons and spinal motoneurons for the treatment of Parkinson's disease or ALS respectively. This presentation will provide a brief update on the successes and remaining obstacles in the field and discuss some of the next steps on the road towards ultimate clinical translation.

O-20 **CONVERTING HUMAN EMBRYONIC STEM CELLS TO DOPAMINE NEURONS FOR THE TREATMENT OF PARKINSON'S DISEASE.**

C.R. Freed, S. Chiba, W. Zhou, and Y-M Lee

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Symptoms of Parkinson's disease have been improved by transplantation of fetal dopamine neurons from abortions, but tissue recovery is difficult. Results in patients have shown that transplants can duplicate but not exceed the best effects of L-DOPA. In people with a history of L-DOPA-induced dyskinesias, transplants can produce similar excess movements. Some patients have come to autopsy. Results show that implanted dopamine neurons can survive indefinitely even without immunosuppression. The fact that fetal tissue is almost impossible to obtain has limited transplants to very few patients worldwide and only two controlled clinical trials, Human embryonic stem cells may provide an unlimited cell source if they can be converted to dopamine neurons, be purified to a uniform cell preparation, and survive implantation into brain. A refined source of dopamine neurons would make it possible to regulate tissue dose and thereby to investigate systematically cell transplantation for Parkinson's disease. We and other groups have studied the differentiation of human embryonic stem cells to a dopaminergic phenotype. The general strategy has been to recapitulate normal brain development. Cells are guided toward a midbrain neuroprogenitor fate and thereafter to ventral mesencephalic dopamine neurons. Sonic hedgehog, FGF-8, and other factors are used. We have found that the BMP-antagonist Noggin can increase the number of dopamine neurons produced *in vitro* and the number that survive transplantation into a rat model of Parkinson's disease. A major problem has been tumor development from residual progenitor cells. To purify dopamine neurons from undifferentiated cells, we have developed mouse embryonic stem cells with GFP knocked into the dopamine transporter. Mice created with these ES cells have GFP+ dopamine neurons which we have FACS isolated. Microarray analysis has demonstrated novel surface markers. Antibodies to these markers are making it possible to purify dopamine neurons differentiated from human embryonic stem cells.

O-21 **Repair And Regeneration Of The Injured Spinal Cord: Opportunities For Clinical Translation Of Basic Research Discoveries**

M. G. Fehlings

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While SCI remains a daunting challenge for clinicians and researchers, many discoveries have been made in the past two decades which could be applied to minimize the impact of secondary injury and promote neural regeneration. This talk will briefly summarize recent advances in our understanding

of the pathophysiology of SCI and discuss the application of this enhanced knowledge in the context of several ongoing or planned clinical trials of potentially key neurosurgical impact including: the STASCIS trial, which is examining the impact of early surgical decompression; a planned clinical trial which will evaluate the neuroprotective efficacy of the sodium/glutamate antagonist riluzole; and the Cethrin trial of a recombinant Rho inhibitor to reduce cell death and enhance neural regeneration(Baptiste and Fehlings, 2006; Fehlings and Perrin, 2006; Baptiste and Fehlings, 2007). In addition, late-stage preclinical studies focusing on neural stem cell based remyelination strategies will be briefly reviewed given the strong translational potential of this approach(Karimi-Abdolrezaee et al., 2006; Eftekharpour et al., 2007).

Selected References:
Baptiste DC, Fehlings MG (2006) Pharmacological approaches to repair the injured spinal cord. J Neurotrauma 23:318-334.
Baptiste DC, Fehlings MG (2007) Update on the treatment of spinal cord injury. Prog Brain Res 161:217-233.
Eftekharpour E, Karimi-Abdolrezaee S, Wang J, El Beheiry H, Morshead C, Fehlings MG (2007) Myelination of congenitally dysmyelinated spinal cord axons by adult neural precursor cells results in formation of nodes of Ranvier and improved axonal conduction. J Neurosci 27:3416-3428.
Fehlings MG, Perrin RG (2006) The timing of surgical intervention in the treatment of spinal cord injury: a systematic review of recent clinical evidence. Spine 31:S28-35; discussion S36.
Karimi-Abdolrezaee S, Eftekharpour E, Wang J, Morshead CM, Fehlings MG (2006) Delayed transplantation of adult neural precursor cells promotes remyelination and functional neurological recovery after spinal cord injury. J Neurosci 26:3377-3389.

POSTER PRESENTATIONS

P-1 **Mp4- And Mog:35-55-Induced Eae In C57bl/6 Mice Differentially Targets Brain, Spinal Cord And Cerebellum Involving Distinctive Patterns Of De- And Remyelination**

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Experimental autoimmune encephalomyelitis (EAE) is the oldest and best-characterized model for the human T-cell mediated autoimmune disease multiple sclerosis (MS), yet much needs to be learned about both the pathogenic processes involved, and the therapeutic options. Gene-modified mice are becoming increasingly indispensable for rapid progress with mechanism-oriented studies. While most gene-modified mice are on the C57BL/6 background, presently there are only two EAE models available relying on myelin oligodendrocyte glycoprotein (MOG) peptide 35-55 and proteolipid protein PLP:178-191. To further account for the pathogenic complexity and heterogeneity seen in MS, we have recently introduced MBP-PLP fusion protein (MP4)-induced EAE as a novel and alternative model for C57BL/6 mice. Here we report that MP4-induced EAE displays characteristic differences in CNS histopathology compared to the MOG:35-55 model. While in the latter, the topology of CNS infiltration remained unchanged throughout the disease, in MP4-induced EAE it was dynamic and stage-dependent shifting from the brain to the spinal cord and finally to the cerebellum. In addition, in the MP4 model demyelination occurred early on and persisted into the chronic stage of the disease. On the contrary, MOG:35-55-induced demyelination was transient occurring only in the initial phase of the disease, even though massive infiltration was still present in chronic EAE. Since the cellular infiltrates were quantitatively unchanged, qualitative changes must have occurred leading to its attenuation. Thereby MOG:35-55-induced EAE clearly differs from other EAE models in which axonal damage and permanent loss has been shown. Further studies will be required to establish whether the demyelination in the MP4 model is irreversible and possibly mediated by axonal damage/transection or whether it represents only inflammation-mediated *functio laesa*. If the former proves true, this should be an important extension of

EAE studies in the B6 mouse, addressing mechanisms of transient *functio laesa* type of demyelination vs. permanent axonal loss.

P-2 **Basic Fibroblast Growth Factor (Fgf-2) Is Required For Functional Recovery By Regulating Remyelination In Injured Peripheral Nerves**

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After peripheral nerve injury, Schwann cells undergo dedifferentiation and proliferation. They form bands of Büngner at the lesion site resulting in a promoting environment for axonal regeneration and ensuing remyelination. Extracellular matrix proteins, neurotrophic factors and hormones modulate these cellular processes during peripheral regeneration. One of the relevant neurotrophic factors is FGF-2, which is expressed in motor and sensory neurons as well as Schwann cells of the developing and adult peripheral nervous system. After peripheral nerve lesion, FGF-2 is up-regulated and influences early peripheral nerve regeneration by regulating Schwann cell proliferation. Exogenous application of FGF-2 promoted peripheral nerve regeneration across long gaps. Evidence for the importance of FGF-2 in remyelination of peripheral nerves came from mice with FGF-2 deficiency and mice overexpressing FGF-2. These studies revealed that FGF-2 inhibits the generation of myelin after sciatic nerve lesion (Jungnickel et al., 2004, Moll Cell Neurosci.; Jungnickel et al., 2006, Dev Neurobiol.). However, the functional outcome during regeneration and the mechanism by which FGF-2 regulates myelin thickness were not explored. To elucidate the role of endogenous FGF-2 on functional and structural recovery, we analysized FGF-2 deficient mice two and four weeks after sciatic nerve crush. These mice showed a delayed recovery of motor and sensory function. Injured peripheral nerves of mutant mice revealed increased myelin thickness and axonal size of regenerated axons. To get insights how FGF-2 regulates myelin thickness after lesion, we measured the expression level of myelin proteins and found an increase in protein zero and myelin basic protein levels. These results demonstrate that FGF-2 has a physiological impact on structural and functional recovery during the peripheral nerve regeneration process.

P-3 **Motor Axon Excitability During Wallerian Degeneration**

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The excitability of motor axons in vivo can be studied using the technique of “threshold-tracking”, which allows the strength of a test stimulus to be adjusted by computer to activate a defined fraction of the maximal muscle action potential (CMAP). During recent years, excitability studies were carried out to investigate the pathophysiological mechanisms in a wide spectrum of neuropathies. Nevertheless, it has not been established to which extent changes in ion-channel function occur in axons that are degenerating but still conducting action potentials. The aim of this study was to study the changes in excitability of motor axons during Wallerian degeneration. Since the problem is difficult to address in humans, investigations were carried out in 3-year-old cats, 8-week-old wild-type mice (C57BL/6J HSD) and 8-week-old slow Wallerian degeneration mutant mice (C57BL/6J WldS). We monitored multiple indices of axonal excitability of the tibial nerve at ankle by tracking the plantar CMAPs under anesthesia. In the distal stump, CMAPs could be evoked ~ 17 hours in wild-type mice, ~ 5 days in cats and ~ 1 week in the WldS mice. Prior to loss of CMAPs there were changes in excitability that were similar among the different experimental models: slowed conduction, increased rheobase, “fanning-out” of threshold electrotonus, decreased input conductance, decreased refractory period and increased superexcitability. Our data suggest that marked abnormalities in membrane excitability precede conduction failure in degenerating motor axons. This should be accounted for in the interpretation of “threshold-tracking” studies in neuropathies with ongoing axonal degeneration.

P-4 **Genetic Determinants Of Cns Repair Following Chronic Demyelination In Mice**

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Demyelinated CNS lesions are the pathologic hallmark of multiple sclerosis (MS) and are accompanied by varying degrees of inflammation, reactive gliosis, oligodendrocyte death, axonal loss, complement activation and antibody deposition. Clinical experience with humans who have MS, and work with animal models of demyelinating disease, has demonstrated that significant CNS repair can occur after demyelination, even without therapeutic intervention, but for reasons that are poorly understood, repair often fails or is incomplete. Recently we have investigated the genetic regulation of CNS repair and remyelination in the Theiler's murine encephalomyelitis virus model of demyelinating disease. We have found marked differences in spontaneous repair in different strains of mice ranging from minimal repair and the progressive accumulation of neurologic deficits in B10.Q mice, to extensive spontaneous myelin repair, with axonal and functional preservation in FVB mice. The "reparative phenotype" of the FVB strain is inherited as a dominant trait in outcrosses with the non-repairing B10.Q strain. To better understand the molecular mechanisms of endogenous CNS repair, we have begun to map the genetic loci that are responsible for the reparative phenotype. Using single nucleotide polymorphisms as genetic markers, we have detected four potential quantitative trait loci (QTLs) for CNS repair on chromosome 3 (LOD~8.9), chromosome 9 (LOD~9.5), chromosome 16 (LOD~2.4) and chromosome 18 (LOD~3.0). The mouse genes for UDP galactosyltransferase 8a, an enzyme that catalyzes the final step in the synthesis of galactocerebroside, an abundant glycosphingolipid component of myelin, and Tyk 2, a janus kinase that plays a central role in controlling the T_H1 immune response, present themselves as potential candidate genes for the QTLs on chromosomes 3 and 9 respectively. Relatively little is known about the genetics of CNS repair and these studies will begin to identify the molecular pathways that are central to this poorly understood process.

P-5 **Immunological Aspects Of Axonal And Neuronal Injury In The Demyelinated Cns.**

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A paradigm shift within the field of multiple sclerosis research has led to the current conception of demyelination as a precipitating factor that triggers a cascade of molecular and cellular events culminating in primary axonal injury, neurodegeneration, and loss of neurologic function. Using the Theiler's virus model of chronic demyelination along with genetic deletion, immunodepletion, and adoptive transfer of isolated populations of immune effector cells, we have created an animal model of multiple sclerosis that decouples demyelination from axon injury. We have shown that demyelination is a necessary but not a sufficient condition for the induction of neurologic deficits in this model. Indeed, our current evidence indicates that CD8+ cytotoxic T cells are the critical cellular mediators of axon injury following demyelination and that perforin is the critical molecular effector of such injury. Moreover, our current working hypothesis is that cytotoxic T cell-mediated axonal injury is triggered by immunorecognition of stress ligands upregulated on demyelinated axons as a result of intra-axonal trafficking disruption and dysregulation. Importantly, our preliminary evidence suggests that interfering with CD8+ T cell recognition of such stress ligands may provide a novel therapeutic avenue for the protection of demyelinated axons. As axonal loss is currently irreparable and because no amount of remyelination-inducing therapy will matter in the absence of a substrate to remyelinate, neuroprotection strategies are critical to the eventual repair of neurologic function in patients with multiple sclerosis.

P-6 **Mmp-9 Controls De-Differentiation And Proliferation Of Peripheral Glia Through Erbb Receptor Mediated Activation Of Mek/Erk Pathway**

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Peripheral glia (Schwann cells) head a unique support network of peripheral regeneration. In response to sciatic nerve damage, Schwann cells induce the expression of matrix metalloproteinase MMP-9 as early as 3 hours after injury. Models of MMP-9 knockout sciatic nerve injury display reduced axonal demyelination due to accumulation of myelin basic protein and ineffective immune cell recruitment, yet an overall increase in cell content. The purpose of this study was to determine the role of MMP-9 in Schwann cell activation and proliferation, through the extracellular signal-regulated kinase ERK of the MAPK signaling pathway. Correlative increase in MMP-9 expression and ERK activation was noted in distal and proximal stumps of transected sciatic nerve from 1 hour (Schwann cell activation) to 4 days (Schwann cell proliferation) after injury, predominantly in Schwann cells. In cultured primary Schwann cells, exogenous recombinant human rhMMP-9 application produced an early but transient dose-dependent activation of ERK that was diminished after treatment with a general MMP inhibitor, GM6001. Studies using a series of inhibitors indicate that MMP-9-induced ERK activation is mediated through ErbB (epidermal growth factor like peptide binding) receptor and MEK (MAP kinase kinase) signaling pathway but not PI3K/Akt pathway. *In vivo* BrdU (bromodeoxyuridine) incorporation studies in MMP-9 knockout mice show an increase in Schwann cell proliferation in transected sciatic nerves relative to wild-type mice. Similarly, we find that MMP-9 inhibits proliferation (BrdU incorporation) of cultured Schwann cells that is restored and further promoted with GM6001 treatment. Our data support a model in which MMP-9, signals inhibition of Schwann cell proliferation, through an ErbB receptor-induced activation of MEK/ERK pathway, leading to Schwann cell de-differentiation and axonal demyelination after nerve damage.

P-7 **Astrocytic Glutamate Transporter Expression Is Altered Following *In Vitro* And *In Vivo* Traumatic Brain Injury**

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Regeneration of neural tissue following injury to the central nervous system may be enhanced by limiting the ensuing neurodegenerative cascades. Glutamate excitotoxicity is one of the major mechanisms associated with neurodegeneration following traumatic brain injury (TBI). We investigated expression of astrocytic glutamate transporters (GLAST, GLT-1) following TBI. This was examined *in vitro* in pure astrocyte cultures from 1-2 day old rat pup cortices. Astrocytes were subjected to mild, moderate, or severe rapid mechanical strain injury or a severe excitotoxic 1mM glutamate exposure for 30 minutes. Western blots were performed with antibodies to GLAST and GLT-1 at various times after injury. Following mild injury GLAST expression decreased at 12, 48, and 72 hours post-injury (62%±3.5, 80%±9.9, 78%±2.0 of control; n=3). In contrast, moderate injury caused an increase in GLAST at 48 and 72 hours post-injury (133%±3.9, 166%±27.6 of control; n=3), while severe injury caused an increase in GLAST at 12 and 72 hours post-injury (126%±17.8, 163%±31.9 of control; n=3). Severe glutamate exposure produced an increase in GLAST at 12, 48, and 72 hours post-injury (120%±17.0, 124%±10.1, 198%±85.6 of control; n=3), and a decrease at 24 hours post-injury (85%±9.0 of control; n=3). GLT-1 was not detected *in vitro*. Glutamate transporters were also examined in rats subjected to a moderate lateral fluid percussion TBI. Western blots of GLAST and GLT-1 were performed at 24 hours post-injury. GLAST and GLT-1 expression decreased in the ipsilateral cortex (78%±13.5, 65%±6.4; n=3) as compared to sham controls. No change was evident in the ipsilateral hippocampus. These findings suggest that glutamate transporter expression is altered following TBI in an injury magnitude-dependent manner, and that this may play an important role in the glutamate excitotoxicity following TBI. Hence

glutamate transporters may be a feasible target to reduce neurodegeneration following TBI and possibly enhance regenerative intervention.

P-8 *In Vivo* Trafficking Of Ampa Receptors To The Plasma Membrane Of Spinal Neurons After Tnfα And Spinal Cord Injury

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Glutamate-mediated excitotoxicity is thought to contribute to cell death in a number of central nervous system (CNS) disorders. However, the specific mechanisms regulating excitotoxicity in the CNS *in vivo* are not well-understood. Recent cell culture work suggests that the proinflammatory cytokine tumor necrosis factor-α (TNFα) causes rapid redistribution of the glutamatergic AMPA receptor (AMPAR) from inactive intracellular stores to the plasma membrane where it can increase a cell's susceptibility to excitotoxicity. TNFα is released after spinal cord injury on a time course that overlaps with excitotoxicity, opening the possibility that AMPAR trafficking by TNFα contributes to secondary injury in CNS trauma. Additional support comes from the observation that in vivo delivery of TNFα to the spinal cord potentiates AMPAR-mediated cell death. However, direct evidence of AMPAR trafficking in spinal cord injury has remained elusive because standard *in vitro* assays such as live cell imaging and surface receptor biotinylation cannot be utilized *in vivo*. The present study overcomes this technical problem by using a combination of techniques including biochemical fractionation, sequential confocal and deconvolution imaging of fixed tissue, automated digital image analysis, and covariance analysis to evaluate AMPAR trafficking *in vivo* after both TNFα injection and spinal cord injury. The findings provide converging evidence that both TNFα and spinal cord injury rapidly increase AMPA receptor levels in the plasma membrane of neurons *in vivo*. This trafficking effect was specific for receptors lacking the GluR2 subunit, an AMPAR subpopulation that has increased Ca^{++}-permeability and greater excitotoxic capacity. Together the data suggest that TNFα-induced AMPAR trafficking occurs as part of the pathological sequelae in spinal cord injury. This process may represent a novel therapeutic target for spinal cord injury treatment.

P-9 Enhanced Growth Of Neurites From Dissociated Dorsal Root Ganglion Neurons Following Spinal Cord Injury

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Spinal cord injury (SCI) is associated with major alterations of sensory function, including sensory deficits and persistent pain. Mechanistic studies of SCI-induced sensory alterations have focused primarily on central neurons and axons, especially within the spinal cord. Various observations, however, suggest that primary sensory neurons with somata in dorsal root ganglia (DRG) may undergo intrinsic functional changes that contribute both to sensory problems and to adaptive sensory responses following SCI. We have asked whether SCI enhances the intrinsic growth state of DRG neurons. Female rats received a spinal contusion at T10. Three days later, DRGs were excised just above, just below, rostral to (C7), and caudal to (L4) the site of injury. Neurons were dissociated and cultured at low density. After 24 hours the cells were fixed and imaged for blind morphometric analysis. In these initial studies, no significant differences were found in the fraction of neurons exhibiting neurites, except for T9 neurons after SCI, which were more likely to have neurites than T9 neurons from naïve animals. Significant elongation was found for the longest neurite measured in each neuron from T9 and T11 in SCI rats

compared to corresponding neurons from naïve rats. Interestingly, sham treatment resulted in neurite lengths that were intermediate between those from SCI rats and naïve rats. Albeit not statistically significant, neurite lengths also tended to be longer in L4 neurons following contusion at T10. These observations suggest 1) that SCI 3 days earlier enhances neurite growth from DRGs just above and just below (and perhaps caudal to) the injury site, 2) that sham surgery (perhaps via inflammatory signals) also can enhance neurite growth, and 3) that SCI acts like a "conditioning lesion" (e.g., Smith and Skene, J Neurosci 17:646, 1997), inducing an intrinsic state in DRG neurons that promotes elongating growth.

P-10 **Thrombospondin-1 Expression Is Increased After Traumatic Brain Injury**

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Recent studies have demonstrated that thrombospondin (TSP)-1, an extracellular matrix protein produced by astrocytes, can promote synaptogenesis (Christopherson et al., 2005). We have shown that TSP-1 expression is upregulated by extracellular ATP through activation of P2Y receptors coupled to several protein kinase signaling pathways (Tran and Neary, 2006). However, little is known about mechanisms regulating TSP-1 expression after traumatic brain injury (TBI). By using an *in vitro* model of CNS trauma which stimulates release of ATP, we found that TSP-1 expression increased after mechanical strain in primary cultures of rat cortical astrocytes. This injury-induced TSP-1 expression involved purinergic signaling since its expression was completely blocked by pyridoxalphosphate-6-azophenyl-2'-4'-disulphonic acid, a P2 receptor antagonist. In addition, the injury-induced expression was attenuated by inhibition of p38/MAPK and Akt, thereby indicating a role for protein kinase signaling in TSP-1 expression induced by trauma. To confirm the results of our *in vitro* data, *in vivo* experiments were performed using a moderate parasagittal fluid percussion injury model. Immunofluorescent staining of TSP-1 in the overlying cerebral cortices showed robust TSP-1 immunoreactivity in rat one and three days post-TBI when compared to sham animals. We conclude that TSP-1 expression after injury can be regulated by activation of P2 receptors coupled to protein kinase signaling pathways and suggest that purinergic signaling may be an important factor in cell-matrix and cell-cell interactions mediated by TSP-1 such as those occurring during CNS repair.

P-11 **Statin-Induced Inhibition Of The Rho Pathway Decreases Cspg Expression.**

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Spinal cord injury (SCI) activates the rho pathway within neurons. SCI-induced activation of the rho pathway inhibits axon growth, while pharmacological inhibition of this pathway stimulates axonal regeneration (Dergham et al., 2002). Recent studies have shown that a commonly used inhibitor of this pathway, Y27632, causes astrocyte activation and increased chondroitin sulfate proteoglycan (CSPG) expression in cultured astrocytes as well as in a rat model of SCI (Chan et al., 2007). Statins (3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors) inhibit the synthesis of cholesterol, but also block synthesis of isoprenoids that transport rho to the cell membrane, a step required for rho function (Hori et al., 1991; Bonetti et al., 2003). Since statins and Y27632 both inhibit the rho pathway, but via different mechanisms, we were interested to see if statins had similar effects on astrocyte activation and CSPG expression. Post-natal rat astrocytes treated with simvastatin for 48 h showed a decrease in CSPG immunoreactivity. Western blot analysis on astrocyte lysates treated with either simvastatin or atorvastatin also showed a decrease in CSPG expression, with no significant change in GFAP levels. These data agree with previous *in vivo* data showing that statins decrease CSPG levels in SCI (Holmberg et al., 2006). The differences in the drug-induced effects on CSPG expression seen here

underscore the need for caution and close attention to the multitude of the effects of any therapy being used for SCI.

P-12 **Epha4 In Spinal Cord Injury And Repair**

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One of the aspects of spinal cord injury (SCI) response that we need to further understand is the mechanism of the interactions between axons and cells of the astroglial/meningeal scar. EphA4, a promiscuous member of the EphA family of repulsive axon guidance receptors, is expressed by multiple cell types in the injured spinal cord, including astrocytes and neurons. We are using a knockout (KO) mouse model to examine the role of EphA4 on scar formation and axon regeneration of the corticospinal tract (CST) in a dorsal hemi-section model of spinal cord injury. In accordance with published data, we confirmed aberrant re-crossing of axons of the corticospinal tract, which is associated with a hopping gait in uninjured animals that precludes behavioral analysis of these mice after SCI. Preliminary data with anterograde tracing suggests increased axonal regeneration of the CST across the lesion site. In contrast, we do not observe a dramatic decrease of glial fibrillary acidic protein (GFAP) expression in the scar. However, other aspects of scar formation might be altered in the mutant. To elucidate the role of EphA4 in different cell types, *in vitro* studies are underway to compare the interactions between wild type and EphA4 KO astrocytes, meningeal fibroblasts and neurons. In addition, we are developing an inducible and cell type-specific gene deletion model of EphA4. Understanding the role of EphA4 in scar formation and axon regeneration may aid in the development of effective therapies for SCI.

P-13 **Strong Adhesion Identifies Potential Neurite Extension And Polarization Sites In Pc12 Cells**

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The dynamic process of neuronal polarization involves the development of neurites into an axon and dendrites. Important intracellular mechanisms have been identified during this process, including actin assembly and disassembly in the growth cone, microtubule dynamics, and cellular tension. It has been suggested that these mechanisms may play a role in axonal specification; however, little attention has been placed on cell spreading and adhesion occurring prior to neurite extension. Here we begin to describe the role of strong adhesion and spreading during the initial polarization stages for PC12 cells, which in the presence of neurotrophic factors, are capable of differentiating into sympathetic-like neurons. We evaluated initial attachment, cell-substrate adhesion, and spreading dynamics of PC12 cells on collagen coated substrates using Interference Reflection Microscopy, and developed an image process algorithm to measure cell spreading area and strong adhesion zones during initial neurite outgrowth. A correlation between zones of adhesion and neurite development was identified to occur in three different stages during the first 90 minutes of spreading. The first stage occurs during initial spreading before neurites have developed, and when zones of adhesion are localized within the cell body. Spreading boundaries reveal anisotropic growth, with a rate of growth of 0.26 (μm^2/min) during this early stage. Total spreading area continues to increase during stage two, with a similar speed. Neurites begin to develop and a competition effect is observed, where strong adhesion occurs at multiple neurite anchoring sites. The level of adhesion appears to specify which neurite develops versus those which retract. In stage three, maximum spreading area is achieved, and polarization appears to begin, with strong adhesion localized at the site of polarization. This preliminary data suggests an importance in adhesion dynamics during both neuronal spreading and polarization.

P-14 **Injection Of Chondroitinase Abc Caudal To A Spinal Cord Injury Promotes Limited Plasticity**

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Recently, the functional recovery seen following chondroitinase ABC (ChABC) treatment of a spinal cord injury site has been partly attributed to increased plasticity of intact systems caudal to the lesion. We hypothesized that injecting ChABC caudal to an injury alone would increase plasticity into denervated regions that may contribute to improved function. Either vehicle or ChABC (50U/mL) was microinjected ipsilaterally at C6 following a C5 complete hemisection (Hx). At 10 days following injury, ChABC had widely digested CSPGs within tissue caudal to the injury, and significantly fewer neurons in the ventral horn were encapsulated by CSPG-rich perineuronal nets after ChABC treatment. While there was a global decrease of ipsilateral serotonergic immunoreactivity at C6 in both vehicle- and ChABC-treated animals, numerous 5HT+ fibers were found throughout ipsilateral grey matter in both groups. Similar numbers of 5HT+ fibers were found in caudal, ipsilateral ventral horn or intermediate grey with ChABC or vehicle treatment, but significantly more 5HT+ fibers were found in the ipsilateral dorsal horn in ChABC-treated animals. In another group of C5Hx animals, ChABC or vehicle was injected ipsilaterally throughout C6-C8, and the animals were assessed weekly in an open-field locomotor test and a grid-walking test. ChABC did not enhance the spontaneous, partial functional recovery observed in both groups. Thus, injecting ChABC caudal to an injury site only does not sufficiently augment the inherent plasticity of intact pathways to have functional consequences. Supported by NIH NS26380 and the Christopher and Dana Reeve Foundation.

P-15 **The P75 Neurotrophin Receptor Inhibits Neurotrophin-Mediated Regeneration And Plasticity Following Spinal Deafferentation**

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Following spinal deafferenation, sensory axons fail to spontaneously regenerate into the central nervous system (CNS), and compensatory sprouting of uninjured axons within the spinal cord is limited. Underlying factors contributing to both the failure of axonal regeneration and the restriction of axonal plasticity include the presence of inhibitory molecules such as myelin-associated inhibitory proteins (MAIPs), and the inadequate supply of neurotropic and neurotrophic support. Since both MAIP and neurotrophin signalling converge on the p75 neurotrophin receptor ($p75^{NTR}$), we hypothesized that $p75^{NTR}$ plays a central role in the inhibition of axonal regeneration and plasticity. When active, $p75^{NTR}$ stimulates RhoA, a small GTPase that reduces actin turnover and leads to growth cone collapse. In the CNS, degenerating oligodendrocytes express MAIPs, which activate $p75^{NTR}$ and stimulate RhoA. Neurotrophins, such as nerve growth factor (NGF) and neurotrophin-3 (NT-3), enhance axonal growth via tropomyosin-related tyrosine receptors (Trks), and prevent RhoA activation via $p75^{NTR}$. We investigated the role of $p75^{NTR}$ by examining primary afferent axonal regeneration across the dorsal root entry zone (DREZ) and intraspinal plasticity following cervical dorsal root injury in wild-type ($p75^{+/+}$) and $p75^{NTR}$ knockout mice ($p75^{-/-}$). In $p75^{+/+}$ mice, subpopulations of primary afferents regenerated within the PNS up to the DREZ, but failed to re-enter the CNS. Conversely, injured primary afferents spontaneously penetrated the DREZ in $p75^{-/-}$ mice, evident at 7 and 28 days post-injury, resulting in the functional re-innervation of the dorsal horn. Regeneration of several afferent populations was prevented in the $p75^{-/-}$ mice when treated with neurotrophin-antagonists (soluble Trk-Fc). Additionally, intraspinal sprouting of bulbospinal neural populations, as well as, uninjured afferents was greater in the $p75^{-/-}$ mice in comparison to $p75^{+/+}$ mice. Given these results, we concluded that the antagonism of $p75^{NTR}$ dis-inhibits neurotrophin-mediated axonal regeneration across the PNS:CNS interface and intraspinal plasticity following spinal deafferentation.

P-16 **Characterization Of PPAR-δ Expression In The Spinal Cord After Injury**

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Peroxisome proliferator-activated receptor-δ (PPAR) belongs to a family of nuclear hormone receptors involved in lipid and glucose metabolism, cell proliferation and differentiation. It is the most prevalent form of PPAR isotype in the central nervous system, where it is involved in brain lipid metabolism, differentiation of oligodendrocyte progenitor cells (OPC) and myelination. It also appears to play a protective role in certain disease models like experimental autoimmune encephalomyelitis (EAE), Parkinson's and cerebral ischemia. Thus, in this study we used a rat spinal contusion model to investigate whether PPARδ expression is changed after spinal cord injury (SCI). Contusive SCI was performed at the T8 level on Sprague-Dawley rats and spinal cord tissue was collected at 1 -28 day post injury (dpi); uninjured spinal cords served as controls. Cross-sections spanning the injury site were used to examine the number and distribution of cells expressing PPARδ. It was observed that after injury, PPARδ+ cell numbers gradually increased throughout the first week. By 7dpi, expression levels were significantly elevated and reached almost twice that of uninjured controls. Cell numbers remained elevated through at least 28 dpi. PPARδ was localized primarily in neurons, NG2+ cells (putative oligodendrocyte progenitors) and astrocytes. This rise in expression could reflect a potential endogenous neuroprotective mechanism that may influence cell survival and promote myelination. Supported by NS043494 and P30-NS045758.

P-17 **Transcriptomic And Proteomic Analysis Of Spinal Microvascular Endothelial Plasticity Following Focal Ischemic Spinal Cord Injury**

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Microvascular dysfunction is a critical pathology that underlies the evolution of secondary injury mechanisms following traumatic spinal cord injury (SCI), although little is known of the molecular regulation of endothelial plasticity observed acutely post-injury. We hypothesize that a significant amount of endothelial dysfunction observed is the result of ischemic/reperfusion events associated with traumatic SCI. Thus, the primary goal of this study was to identify novel molecular regulators of endothelial dysfunction specifically associated with the ischemic component of traumatic SCI. Female Sprague-Dawley rats were subjected to focal spinal cord ischemia by micro-application of endothelin-1 (ET-1; 5 pmol) to the penetrating branches of the anterior spinal artery. At 2 and 5 days post-ischemia, microvessels were highly enriched from affected spinal tissue by intravascular lectin perfusion and FACS purification. RNA and protein were extracted and used for focused microarray (84 total endothelial cell biology-related mRNAs) and proteomic analyses, respectively. At both time points, temporally-specific dysregulation of genes regulating angiogenesis and endothelial cell function were observed. More specifically, at the 2 day time point, mRNAs encoding for molecules involved in regulation of permeability and vascular tone as well as the inhibition of angiogenesis were significantly down-regulated but were unchanged at 5 days post-ischemia. Conversely, at 5 days post-ischemia, genes potentially involved in the inhibition of the endogenous angiogenic response and production/proteolysis of extracellular matrix were significantly induced as compared to the early time point. Proteomic validation of the dysregulation of these specific mRNAs as well as the identification of other pathologically regulated endothelial cell proteins is ongoing. These results implicate multiple novel molecular regulators of spinal endothelial dysfunction, several of which may have potential as therapeutic targets for a variety of disorders including spinal trauma, stroke, and neurodegenerative diseases having a vascular component. This

research was supported by NS045734, RR15576, Norton Healthcare (SRW), and the University of Louisville School of Medicine (RLB).

P-18 **Gene Expression In Lateral Vestibular Neurons Axotomized By A Cervical Spinal Cord Injury**

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Gene expression by neurons is altered following axotomy but changes in patterns are incompletely understood as most studies have used in situ hybridization on tissue sections, thereby limiting the number of genes that can be evaluated at different post injury intervals. We used microarray technology to identify changes in gene expression in lateral vestibular neurons that were isolated by laser micro dissection 4 hrs to 42 days after a cervical hemisection lesion that unilaterally disrupts vestibulospinal axons. Our goal was to identify coherent classes of genes that are differentially expressed as a result of axotomy. Isolated lateral vestibular neurons (>100/rat) were processed for total RNA and mRNA was amplified linearly and processed on Affymetrix rat 2.0 chips. Microarray data were supplemented by quantitative PCR (Q-PCR) to confirm gene identity. Results were processed using RMA in Bioconductor, differential expression was analyzed by Limma and gene function was identified from the Gene Ontology database. Few changes either up or down were observed at 4 hr, but expression of large numbers of genes changed from 24 hr through 42 d, with the most dramatic alterations between 24 and 48 hr postoperatively. In general early changes were found in the expression of nuclear transcription factors (JUN, RET and others), genes associated with the plasma membrane and those involved in cell signaling processes such as G-protein and signal transduction genes; later changes occurred at 3d with genes related to the cytoskeleton and biogenic amines, at 7 and 14 d for tubulins, myosin, chromatin and metabolic processes (decreased glycolysis, energy metabolism and fatty acid synthesis). Detailed sequences of gene expression in neurons at different stages of injury will provide baseline data for evaluating the effects of acute or delayed interventions that are designed to promote regeneration. Supported by New York State Spinal Cord Research Program and the Shrine Hospital for Children, Philadelphia.

P-19 **The Expression Of Genes In Distinct Populations Of Cells After Spinal Cord Injury And Exercise**

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Both spinal cord injury (SCI) alone and with exercise results in behavioral and anatomical plasticity in the spinal cord below the level of the lesion. This plasticity may be due to the responses of numerous cell types. For instance, it is likely that increased expression of trophic factors contributes to anatomical and synaptic remodeling such as sprouting of dorsal root ganglion (DRG) fibers in the dorsal horn and intermediate grey of the spinal cord. In an attempt to elucidate the mechanisms by which exercise benefits SCI recovery, we analyzed the expression of genes related to plasticity in separate regions and cell types in the spinal cord grey matter following injury and exercise. 10 adult rats received a thoracic level 10 spinal cord transection. 5 days after injury, five of these rats began a 5 day passive cycling regimen. Following exercise, the lumbar enlargement (L4-L6) and corresponding DRG's of normal, injured only, and injured plus exercise animals were removed and frozen for sectioning. Immunohistochemical methods and laser micro dissection were used to collect motoneurons, astrocytes, and regions of intermediate grey for mRNA isolation to be used for relative expression analysis by real-time PCR. The beneficial effects of short-term exercise at the gene level include reduced expression of extracellular matrix molecules in astrocytes that contribute to inhibition of repair. Furthermore,

motoneurons upregulate expression of neurotrophic factor receptors as well as factors related to cell survival. Further analysis of the response of different cell types following injury and exercise will measure changes in the expression of trophic factors and receptors in cells of the intermediate grey matter, genes involved synaptic remodeling by motoneurons, and regeneration associated genes by the neurons of the DRG. Supported by NIH NS26380.

P-20 **Functional Genomic Analysis Of Retinal Ganglion Cells Purified From Wlds Mice**

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Axon degeneration is an active process, and is a characteristic feature in many neurodegenerative diseases including multiple sclerosis, ALS and glaucoma, but the molecular mechanisms that control axon self-destruction remain unclear. Remarkably, axons of the slow Wallerian degeneration (Wlds) mice survive several weeks after nerve transection, and this axon specific protection is intrinsic to the neuron and distinct from caspase-3 dependent apoptosis. The mutant Wlds mutation encodes a chimeric fusion protein consisting of UBE4B, an ubiquitin assembly factor, and NMNAT1, a nuclear enzyme involved in synthesis of NAD+, and it is thought that the full axon protective effect requires both components of Wlds gene. Interestingly, the Wlds protein product is localized primarily in the nucleus and suggests that it may confer distal axonal protection through early transcriptional control of axonal protein expression or trafficking preceding nerve injury. To investigate whether axon protection by Wlds is transcriptionally dependent, we took advantage of our ability to highly purify retinal ganglion cells (RGCs) to profile the differential gene expression of RGCs from Wlds and wildtype mice. We performed expression analysis and confirmed the differential expression of the top upregulated and downregulated genes by RT-PCR. We identified a small number of genes (<0.1% of total RGC transcriptome) that reproducibly change more than 2-fold in either direction, providing a highly specific list of candidate genes. Using RGC aggregates, which grow axon bundles in culture that can easily be transected, we have established an in vitro assay for assessing the necessity or sufficiency of the identified genes for axon degeneration. We hope that one or more of the genes identified in this screen may lead to better understanding of the mechanism of Wlds axon protection and how axon degeneration can be prevented in nervous system injury and disease. Support from the National Eye Institute, NIH Medical Scientist Training Program (MEV) and HHMI Medical Research Fellowship Grant (JTW).
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P-21 **Transplanted Type-2 Astrocytes Derived From Embryonic Glial Precursors Fail To Support Functional Recovery After Spinal Cord Injury And Promote Neuropathic Pain**

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We have previously shown that type-1 astrocytes (T1 GDAs) derived from embryonic glial restricted precursors (GRPs) can suppress scar formation, support axon regeneration, enhance neuroprotection and promote robust functional recovery when transplanted into acute spinal cord injuries in adult rats (http://jbiol.com/content/5/3/7). In addition to T1 GDAs, embryonic GRPs are also able to generate astrocytes of a type-2 phenotype in vitro (T2 GDAs). A question remained therefore as to whether type-2 GDAs would be of similar benefit when transplanted into the injured spinal cord i.e. are all astrocytes generated from embryonic precursors equivalent? We have therefore conducted a direct comparison of the potential benefits of transplanting purified populations of T1 versus T2 GDAs into acute spinal cord injuries in adult rats. In sharp contrast to T1 GDA treated cords, sensory axons failed to cross dorsal column injuries bridged with T2 GDAs. Similarly, Grid Walk analysis of rats receiving transplants of T2 GDAs into rubrospinal tract injuries showed no recovery of locomotor function. Rats receiving T1 GDAs reached pre-injury Grid Walk scores by 28 days post injury. Intra-lesion transplants

of T2 GDAs also failed to suppress neuron atrophy in the red nucleus. Transplants of T2 GDAs did however promote the onset of mechanical allodynia and thermal hyperalgesia at 2 weeks post injury, an effect that continued to intensify at time points up to 5 weeks post injury. Importantly, rats receiving T1 GDAs did not increase mechanical or thermal sensitivity above pre-lesion values. Our data clearly demonstrates that not all astrocytes that can be derived from embryonic glial precursors are equivalent in their ability to repair the injured adult CNS. Generation of the right types of astrocytes such as T1 GDAs is therefore vitally important in considering the use of neural or glial precursors for repair of the injured adult central nervous system.

P-22 **Guanosine Improves Function Associated With Reducing Apoptosis And Inflammation After Spinal Cord Injury In Rats**

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Spinal cord injury results in progressive waves of secondary injuries, cascades of noxious pathological mechanisms that substantially exacerbate the primary injury and the resultant permanent functional deficits. Secondary injuries are associated with inflammation, excessive cytokine release, and cell apoptosis. The purine nucleoside guanosine has significant trophic effects and is neuroprotective, anti-apoptotic in vitro, and stimulates nerve regeneration. Therefore, we determined whether systemic administration of guanosine could protect rats from some of the secondary effects of spinal cord injury, thereby reducing neurological deficits. Systemic administration of guanosine (8mg/kg/day, i.p.) for 14 consecutive days, starting 4 hours after moderate spinal cord injury in rats, significantly improved not only motor and sensory functions, but also recovery of bladder function. These improvements were associated with reduction in the inflammatory response to injury, reduction of apoptotic cell death, increased sparing of axons and preservation of myelin. Our data indicate that the therapeutic action of guanosine probably results from reducing inflammation resulting in the protection of axons, oligodendrocytes and neurons, and from inhibiting apoptotic cell death. These data raise the intriguing possibility that guanosine may also be able to reduce secondary pathological events and thus improve functional outcome after traumatic spinal cord injury in humans.

P-23 **Characterization Of Spinal Cord Activity During Task And Rest Using Functional Magnetic Resonance Imaging**

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Understanding healthy spinal cord function is necessary before altered or impaired spinal cord activity can be studied. Recent advances in functional magnetic resonance imaging (fMRI) of the spinal cord have made possible the investigation of the neuronal response in the spinal cord to various stimuli. This study aims to characterize the neuronal response detectable by fMRI of the spinal cord in response to noxious stimuli and during rest. Twenty volunteers participated. The noxious stimulus was a heat stimulus applied tonically and oscillating with a thermode (average temperature of 47°C) to the volar forearm, whereas at rest the thermode was set to skin temperature or was removed. A block paradigm with a total of 42 volumes was used with a spin echo sequence. The two rest conditions were not significantly different (4.93% thermode removed and 5.01% thermode at skin temperature); this is a good indication that the presence of the thermode is not contributing significantly to the signal change. Average percent signal change recorded from the noxious stimulus conditions were 5.45% for tonically applied heat and 5.40% for oscillating heat. The pain conditions show significantly greater signal change than the rest condition ($p<0.001$). Paired samples t-tests were performed to look for trends in the distribution of activity. Neuronal activity in spinal cord segments C4, C5 and C6 show significantly more active voxels than segments C7, C8, and TI ($p<0.05$) during the tonically applied noxious stimulus but

not during the oscillating heat. No trend in the distribution of activity was seen during rest. Characterizing the neuronal response in the spinal cord in response to stimuli and in the absence of stimulation, as detected by fMRI, is a key step toward the use of this tool for the investigation of impaired spinal cord function.

P-24 **Quantitative Gait Analysis In Rats After Experimental Stroke**

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Gait impairment is commonly observed among patients with stroke. Although gait changes after stroke are well-characterized in humans, gait data in quadrupeds after stroke are scarce. To assess the effect of ischemic stroke and environmental enrichment (EE) on functional ambulation in rats, we quantify temporal gait changes using the *CatWalk*™ automated gait analysis system. While traversing on a one-meter long glass walkway, the *CatWalk*™ recorded the images of rat footprints came in contact with the glass floor. Ischemic stroke was induced via temporary distal middle cerebral artery occlusion (MCAO) for 60 minutes. Rats were housed either in the standard or enriched environment at one week after MCAO for a period of 4 weeks. They were subjected to 3 consecutive trials of gait analysis each at 4 days and 5 weeks after MCAO or sham-operation. We found that the intensity, maximal contact area and angle were significantly decreased in the affected forepaw at 4 days after MCAO, the relative print positions between the fore and hindpaws and limb coupling were also affected, suggesting that stroke not only affected the sensorimotor status but also reduced the coordination between limbs. Except for the paw angle, the above-mentioned impairments persisted for at least 5 weeks of time after MCAO. Rearing in the enriched environment significantly improved the paw contact area and relative print position of the affected limb in ischemic rats compared to standard environment. Contrary to the gait changes described in human stroke patients, temporal parameters such as swing and stance time were not affected by MCAO in rats. In conclusion, the *CatWalk*™ gait analysis is a useful tool to measure gait related motor function changes in ischemic quadrupeds. Further, EE reduces post stroke gait impairment in the adult rats.

P-25 **Real-Time Video Motion Analysis For Assessment Of Rat Limb Function Following Nerve Injury And Repair**

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The aim of the study was to develop reproducible functional evaluation methods for rat limb nerve injury models using video motion analysis. Six rats per group underwent sciatic, tibial, peroneal, median, ulnar, radial, and combined median and ulnar nerve transection. Measurements included compound muscle action potential (CMAP), somatosensory evoked potential (SEP), grip strength, sciatic function index and upper limb functional index. Gait cycle was analyzed using video recording of wrist and metacarpophalangeal (MP) joint movement in the upper limb or ankle movement in the lower limb. Limb-appropriate changes of toe spread were simultaneously recorded during the same gait cycle. All measurements were conducted preoperatively and 1, 3, 4, 6, 8 10, 12 and 16 weeks postoperatively and compared with sham and non-operated controls. CMAPs in appropriate muscles were reproducibly measured after proximal, near-nerve stimulation with needle electrodes. Grip strength was not impaired by radial nerve injury. Combined median and ulnar nerve injury led to the largest reduction in grip strength that didn't recover until 12 weeks postoperatively. Motion analysis quantified changes in wrist and MP joint extension following all types of upper limb nerve injury. Similarly in the lower limb, motion analysis reliably measured change in ankle-angle with time and discriminated well between the different

types of nerve injury. Changes in toe spread were only observed after combined median and ulnar nerve or after radial nerve injury in the upper limb and was less sensitive to change in the lower limb. Nerve injuries of the upper and lower limb in rats can be reliably evaluated by combining electrophysiology, behavioral tests and motion analysis. This approach has been used to compare recovery of function after nerve repair using a variety of single and multi-channel biodegradable polymer scaffolds.

P-26 **Age-Associated Deficits Following Sci**

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Most spinal cord injuries (SCI) occur in young adults. In the past few decades, the average age at time of SCI and the percentage of injuries in persons over the age of 60 have increased. Studies have shown that there is an age-associated delay in the rate of remyelination following toxin-induced demyelination of the spinal cord, suggesting that there may be an age-associated difference in regenerative efficiency. Here we examine locomotor recovery, bladder recovery, and myelin pathology in young (3 months), aged (12 months), and geriatric (24 months) female rats following contusion SCI. Our assessments indicate that aged and geriatric rats have a delayed rate of locomotor recovery following contusion SCI as compared to young rats. Additionally, aged and geriatric rats have significantly slower bladder recovery as compared to young rats. Examination of myelin pathology reveals that aged and geriatric rats have significantly greater area of pathology and amount of demyelination, as well as significantly less remyelination as compared to young rats following contusion SCI. These data suggest that there is an age-associated decline in the rate and extent of both locomotor and bladder recovery, and that age adversely affects the degree of pathology, demyelination, and remyelination that results following contusion SCI.

P-27 **L-Dopa-Induced Dyskinesias In Aged And Non-Aged 6ohda Lesioned**

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A serious problem Parkinson's disease patients face with the long-term use of L-DOPA therapy is the development of dyskinesias. The phenomenon has been studied extensively, and yet there is limited research that explores age as a causative variable in animal models (Tamas *et al.*, 2005). We investigate the effect of age on the susceptibility and severity of L-DOPA-induced dyskinesias in unilaterally 6-OHDA lesioned rats. The extent of lesion was evaluated using an amphetamine-induced rotation test. In the amphetamine-induced rotation test, the rate of turning was higher for aged rats in comparison to non-aged rats ($p<0.05$). All rats underwent 21 days of daily L-DOPA and developed recurring L-DOPA-induced abnormal involuntary movements (AIMs). On day one of treatment, aged rats exhibited more severe AIMs ($p<0.05$) and their dyskinetic behavior was significantly longer-lasting than for non-aged rats ($p<0.05$). After 21 days of treatment, both experimental groups exhibited AIMs of similar severity and duration. Threshold tests in animals with established AIMs indicate that manipulation of dopaminergic activity in aged rats is associated with more significant behavioral effects. The lowest L-DOPA dose required to elicit abnormal behavior was determined in animals with established AIMs. Aged rats exhibited a lower L-DOPA threshold compared to the younger rats ($p<0.05$). These studies show that the aged brain is more susceptible to pathologies caused by perturbations in the catecholamine system. Neural repair strategies must consider this difference to insure optimum clinical benefit without unwanted side-effects.

P-28 **Locomotion In Young Adult And Reproductively Senescent Female Rats: Implications For Studies Evaluating Locomotor Behaviour**

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INTRODUCTION: Rats are one of the most commonly used species for assessing behaviour in a variety of models of neurological disease. It is well-known that aging is associated with changes in the neuromuscular system. Given that sensorimotor, especially locomotor, abilities of aged rats compared to young adult rats are incompletely described, and that aging may account for discrepancies between studies using rats of different ages, we set out to determine the locomotor ability of young (Y) compared to middle-aged (MA) adult rats. **METHODS:** Three to 4 month-old (Y; n=7) and 12 to 14 month-old (MA; n=7) female Wistar rats were used. Postural strength was assessed using the inclined plane test. Skilled locomotion was evaluated using the ladder and tapered beam tasks. Flat-ground locomotor abilities were assessed using ground reaction force (GRF) and kinematic measurements. **RESULTS:** Y and MA animals performed similarly on the inclined plane (maximum angle for support (mean+/-SE) for Y=65+/-2 degrees; MA=61+/-1 degrees). Both Y and MA animals use their limbs similarly and make similar numbers of errors while performing the ladder and tapered beam tasks. Both Y and MA rats prefer to trot when locomoting between 60-90 cm/s. GRF analysis revealed that MA and Y rats locomote similarly although MA rats tended to generate more lateral force with their hind limbs. Details of kinematic analysis will be presented. **CONCLUSIONS:** Y and MA rats have similar postural support and locomotor abilities. The tendency for MA rats to generate more lateral force with their hindlimbs should be considered when comparing locomotor differences between aged and young rats. **SUPPORT:** NSERC, UCVM

P-29 **The Effect Of Estrogen On Autonomic And Sensorimotor Recovery In Female Rats Using A Clinically Relevant Spinal Cord Injury Model**

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INTRODUCTION: While improved sensorimotor recovery is an important goal of SCI research, SCI patients desire improvement in other conditions that develop following SCI, including autonomic dysreflexia (AD). AD affects up to 70% of individuals following severe SCI at or above the sixth thoracic vertebral level (T6). AD manifests as uncontrolled severe hypertension during somatic or visceral stimulation below the level of injury. We have previously shown 17β-estradiol to ameliorate AD in SCI male mice. The present study examined the effect of 17β-estradiol on AD and gross sensorimotor recovery in female rats using a clinically relevant SCI model. **METHODS:** Animal groups comprised of adult female Wistar rats that were either: 1) reproductively intact and in proestrus (NonOx) (n=3); 2) ovariectomized + 17β-estradiol (EOx) (n=4); 3) ovariectomized (Ox) + vehicle (n=3). A 50g-calibrated clip model of SCI was used. AD was induced by colorectal distension four weeks after SCI. Postural strength and exploratory locomotor abilities were examined weekly using the inclined plane and BBB rating scale, respectively. **RESULTS:** Mean (+/- SE) changes in MABP during CRD were NonOx=25 (+/- 3) mm Hg; Ox=35 (+/6) mm Hg; EOx =25 (+/- 3) mm Hg. All animals demonstrated impaired postural support following SCI that did not improve over time. BBB scores improved for all animals over the duration of the study. **CONCLUSIONS:** Preliminary results indicate that 17β-estradiol tends to reduce the severity of AD, but does not improve postural support or exploratory locomotor behaviour in female rats using a clinically relevant SCI model. **SUPPORT:** NSERC, UCVM, and O'Brien Centre for BHSc.

P-30 **Theraputic Every-Other-Day Fasting Improves Recovery From Thoracic Spinal Cord Contusion Injury**

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Previously we found that therapeutic every-other-day fasting (fasting started after injury) improved functional recovery, reduced lesion size, increased beta-hydroxybutyrate and full-length/truncated trk B ratio in a cervical spinal cord injury model. In this experiment we tested if therapeutic EODF would improve recovery after a moderate (1.5 mm displacement) low thoracic contusion injury. Four groups of adult male Sprague Dawley rats were studied: Control (ad libitum fed), Post-EODF (therapeutic EODF started after injury), Pre-EODF (EODF started 3 weeks prior to injury), and a Pair-Fed control group (same total grams of food as the EODF groups provided in daily portions). The open field locomotion behavioral test revealed that both the Pre-EODF and Post-EODF groups performed significantly better than either the control group or the Pair-Fed control group. The catwalk device revealed several differences in footprint analysis among groups. The Pre-EODF group had higher regularity index (coordination) than either the control group or the Pair-Fed control group, and the Post-EODF group had a higher score than the control group. A higher percentage of animals in both the Pre and Post EODF groups were within the normal range of fore and hind limb swing duration differences. Additionally, both the EODF groups had significantly smaller abnormalities in hindlimb to forelimb placements. Lesion size and spared host tissue are being accessed and will be reported. In summary, either therapeutic EODF started after injury, or Pre-EODF, can improve functional recovery after a low thoracic spinal cord contusion injury as revealed by multiple behavioral measurements. Therapeutic EODF is a safe and simple treatment that displays robust effects in two different animal spinal cord injury paradigms making it an interesting candidate for clinical translation. Supported by grants from Canadian Institutes of Health Research and Craig H. Neilsen Foundation. (*) = co-authors of poster.

P-31 **Omega-3 Fatty Acid Diet Boosts The Effects Of Exercise On Overcoming Growth Inhibition By MAG And Nogo-A After Traumatic Brain Injury**

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The presence of molecules inhibitory to axonal outgrowth, such as myelin-associated glycoprotein (MAG) and Nogo-A, can be detrimental to the maintenance of normal neuronal function in the adult CNS under challenging conditions such as traumatic brain injury (TBI). Given the benevolent action of physical activity on the brain, we have investigated the potential of voluntary exercise to overcome the growth-inhibitory action of MAG and Nogo-A. In addition, based on the increasingly recognized role of the omega-3 polyunsaturated fatty acid, docosahexaenoic acid (DHA), dietary supplementation on brain function and plasticity, we have investigated the possibility that this dietary supplementation can act together with exercise to boost regeneration after TBI.

Adult rats were subjected to an experimental model of TBI, fluid percussion injury (FPI), applied lateral to the left hippocampus. Rats were exposed to voluntary exercise or a sedentary condition, while subgroups of each were exposed to the effects of DHA diet after FPI for 13 days. Western blot analysis showed that FPI elevated hippocampal levels of MAG and Nogo-A by 65% and 58%, respectively, compared to sham controls. While exercise or the DHA diet by separate reduced the post-TBI increase in MAG and Nogo-A levels. Interestingly, the combined application of exercise and DHA diet after injury further reduced the injured-related increases of MAG and Nogo-A. These results suggest that both exercise and omega-3 fatty acid diet are important for counteracting the effects of brain trauma and that the greatest effect can be achieved by combining the exercise regimen with DHA diet. In conclusion, our findings indicate that exercise and DHA diet can help the recovery processes after TBI by promoting a suitable substrate for growth and repair of neural circuits. (Supported by NIH awards NS45804 and NS50465).

P-32 **Chronic Serotonin Agonist Administration Combined With Exercise Promotes Long-Term Recovery In Spinal Rats**

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We tested the hypothesis that chronic quipazine administration paired with passive or active exercise promotes long term recovery from complete spinal injury. Low doses of quipazine, a 5-HT_2 receptor agonist, improve locomotor performance in spinalized animals because of denervation induced receptor upregulation on spinal neurons caudal to the injury. Exercise prevents hindlimb muscle atrophy allowing for better behavioral expression of these locomotor improvements. Exercise also increases neurotrophin levels in the exercised muscles and spinal cord that promote synaptic plasticity. Rats received quipazine (0.075 mg/kg, IP) paired with exercise starting 2 weeks after spinal cord transection (n=19) compared with rats that received exercise alone (n=7). Different treadmill and passive cycling exercise protocols that each started at 1 week post injury were compared. Behavioral data (BBB score) at 8 weeks post injury showed that chronic quipazine and exercise significantly improved hindlimb motor function (BBB=3.0) vs exercised controls (BBB=1.3), regardless of exercise protocol. Acute quipazine challenge at 8 weeks increased hindlimb motor function (BBB=6.0), but did not reveal any further differences between groups. It should be noted that BBB score may not be sensitive enough to reveal small differences in behavioral expression among exercise protocols. Nevertheless, there are molecular changes at the lumbar spinal cord and lumbar motoneurons that demonstrate exercise related effects. Following one week of acute exercise, changes in mRNA levels of c-fos, HSP-27 and receptors for BDNF and GDNF were found, dependent on exercise protocol, compared to nonexercised controls. These factors are beneficial for synaptic formation, sprouting and reconnectivity, suggesting a potential mechanism by which exercise could contribute to long term recovery in combination with chronic quipazine. Supported by PO1 NS24707, PO1 NS055976, and by two grants from The Craig H. Neilsen Foundation to K.A. Moxon and to J.S. Shumsky.

P-33 **A Novel Nsaid Improves Locomotor Recovery In Rats With Spinal Cord Injury**

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Background: Neuroinflammation plays important roles in locomotor disability secondary to spinal cord injury (SCI). In previous studies using rodent models of SCI, we have obtained evidence that ibuprofen-phosphatidylcholine (IBU-PC) has a moderate neuroprotective efficacy. High doses of Vitamin E (VE) can inhibit phospholipase A_2 (PLA_2), while NSAIDs inhibit cyclooxygenase (COX) activity. The combination of IBU-PC and VE may have synergistic anti-inflammatory effect via inhibition of both enzymes. **Aim:** We hypothesized that IBU-PC / VE, when administered to rats in the acute stage of SCI, could improve locomotor function. **Methods:** Female Sprague-Dawley rats were randomized into: a group that was uninjured; and two groups where SCI was induced at T10 using an Infinite Horizon Impactor with 150 kdyne impacting force and 0 dwell time. The test drugs (either IBU-PC/VE at an NSAID dose of 40 mg/kg or saline) were administered to SCI rats 30 min post-injury via a single bolus injection via the jugular vein. The locomotor function of rats was evaluated using the Basso, Beattie and Bresnahan (BBB) on days 3-35. **Results:** The IBU-PC/VE group had significantly higher BBB score than the saline group over the entire study period ($p<0.05$), with the largest improvement seen on day 14. Changes of eicosanoids at the lesion site of the spinal cord 24 hours post-SCI were evaluated by high pressure liquid chromatography/tandem mass spectroscopy (HPLC/MS/MS) and showed that both PGE_2 and LTB_4 levels were significantly increased several fold post-SCI (vs sham), which was reduced in rats treated with IBU-PC/VE ($p<0.05$ for PGE_2, $p=0.08$ for LTB_4). **Conclusion:** IBU-PC/VE systemically administered 30 min post-SCI shows clear protection of locomotor function. The exact mechanism underlying this phenomenon and the optimal dosing profiles will be explored in future studies. (Supported by Army grant W81XWH-05-0118).

P-34 **A Prediction Model For Determining Over Ground Walking Speed Following Locomotor Training In Motor Incomplete Spinal Cord Injury Patients**

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It has been well established that locomotor training in motor incomplete spinal cord injury patients can result in improved locomotor ability including improved gait speed. Not all patients experience the same magnitude of training effect, however, and it has been difficult to predict which patients will benefit from locomotor training and/or the magnitude of that training effect. To address this question we retrospectively studied 30 patients to determine which of a spectrum of clinical features best predicted walking speed following 3 months of locomotor training. Using a stepwise regression analysis, we designed a predictive model that was then tested on 5 additional subjects in a prospective manner.

Four independent predictors of post-training gait speed were identified: voluntary bowel and bladder voiding, spasticity affecting stance, pre-training walking speed, and the square root of the time since injury. The formula for the model,

Final over ground walking speed = 21.48+ (voluntary bowel & bladder voiding x 18.78) + (spasticity affecting stance x 14.30) + (initial walking speed x 0.87) – (square root time from injury onset x 6.06),

predicts 78 percent of the variance of measured walking speeds following training.
With prospective testing of the model, the predicted walking speeds following locomotor training matched the measured walking speeds within 4.38 ± 2.50 cm/sec which is less than 5% of normal walking speed. Using this model, clinicians should now have a better insight in to which of their motor incomplete SCI patients will benefit from locomotor training and how much.

P-35 **Effect Of Injury Severity And Duration On H-Reflexes In Spinal Cord Injury Patients Stepping In The Lokomat Robot**

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Previously, we demonstrated that recovery of walking in motor incomplete spinal cord injury (SCI) patients following 3 months of robotic body weight support treadmill training (BWSTT) could be related to supraspinal plasticity (Winchester ’05). To address whether the same locomotor training could be associated with plasticity at the level of the spinal cord, we have been studying the effect of robotic BWSTT on the electrical equivalent of the soleus muscle stretch reflex, the soleus H-reflex. The first step in this process has been to determine the effect of injury severity and time since injury on the H-reflex prior to any locomotor training. All classes of SCI patients have been studied during prone lying, standing and at both mid-stance and mid-swing during stepping at 2 speeds in the Lokomat, a robotic gait orthosis.

In control subjects, H-reflexes were minimally diminished in mid-stance during stepping compared to prone lying or standing and more diminished during mid-swing. In motor incomplete SCI patients, H-reflexes were approximately 30% greater than in controls but showed the same pattern of modulation in mid-stance and mid-swing during stepping. Patients with motor complete SCI showed similar H-reflexes as controls during prone lying or standing but those reflexes were markedly diminished in mid-stance during stepping and essentially eliminated in mid-swing. Motor complete patients whose injuries were less than 3 years old did not have as much diminution of their H-reflexes during mid-stance at the slower of the two walking speeds as did motor complete patients whose injuries were of greater duration. These results show that severity of injury, and in some cases, time since injury, affect the magnitude of the H-reflex in SCI patients and that, in the case of motor complete patients, some aspect of stepping in a robotic BWSTT system diminishes spinal cord excitability as estimated by H-reflexes.

P-36 **Retrospective Medical Records Analysis of Olfactory Mucosa Autograft Patients and Comparison to Patients with Rehabilitation Alone or with Other Interventions**

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Olfactory mucosa is a readily accessible source of stem-like progenitor cells and olfactory ensheathing cells. Both of these cell types proved to be useful for spinal cord injury in experimental animal studies. Olfactory mucosa autografts (OMA) are being performed by a team of physicians in Lisbon, Portugal on AIS A or B patients with lesions ranging from 1-7.5 cm present at C3-T11 vertebral levels. The 18 month follow-up of the first 7 patients to receive this OMA procedure was recently published (JSCM 29:191-203, 2006). Thirty-three of the subsequent patients that had OMA surgery performed in Portugal had therapy at the Rehabilitation Institute of Michigan (RIM). Physical therapy notes on these OMA patients and patients with similar injuries in terms of severity and level of injury were examined. The primary goal of this study was to identify any adverse reactions that may relate to the OMA procedure especially in terms of infection, pain, or loss of sensation. A secondary goal was to determine any differences in the progression of OMA patients compared to non-OMA with rehabilitation. All patient comments were noted and judged as being positive, negative or neutral. OMA patients had an average of 48 rehab 3-hour rehab sessions over an average 4 month period. OMA surgery was performed at 10 months after injury in 1 patient, > year after injury in 12 patients, 2-4 years after injury in13 patients, >4 years after injury in 7 patients. The distribution of injury in patients was 7 (AIS A, C3/4-C5), 8 (AIS A, C6-T1), 10 (AIS A, T2-T11), 4 (AIS B, C3-C5), 4 (AIS B, C6-T1). No severe side effects of the OMA procedure were found. The majority of comments by OMA patients were positive. Most of the OMA and non-OMA patients made progress in terms of their selected individual goals.

P-37 **Recovery Of Hindlimb Stepping Following Spinal Cord Injury In The Rat: Comparison Of Open Field Scores And Automated Paw Print Analysis At Different Gait Speeds And Recovery Times**

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Recovery of locomotion following spinal cord injury in the rat has been most commonly characterized by open field locomotor scoring (Basso et al. ’94) and paw print analysis which has recently been automated (Hamers et al. ’01). These measures evaluate different features of stepping but have in common that the gait speeds studied are self-selected by the animal. Recently, a new automated paw print analysis system, the Digigait (Kale et al. ’04), has been developed where paw print video is taken from below through a clear treadmill that can be run at different speeds. As the device makes it possible to evaluate stepping parameters at multiple, standardized gait speeds, we used it to investigate the recovery of hindlimb stepping in Long Evans rats over 7 weeks following a moderate contusion spinal cord injury at T9 by comparing paw print parameters with treadmill speed, open field locomotor scores, and recovery time.
Following spinal cord injury, hindlimb stride length and stride frequency increased with increasing treadmill speed whereas stance width was unchanged across treadmill speeds and stance/swing ratio and hindlimb shared stance time decreased with increasing treadmill speed. Only a few stepping parameters were weakly correlated with open field locomotor scores (stance width>stride length). Likewise, only one stepping parameters was weakly correlated with recovery time (stride length). Stepping parameter to stepping parameter comparisons showed good correlation either across all walking speeds (stance/swing ratio to hindlimb shared stance time and to stride frequency) or within walking speeds (stride length to stride frequency and to hindlimb shared stance time). Analysis of relationships between different hindlimb paw print parameters and anatomical measures of the severity of spinal cord injury are ongoing. This work shows how paw print analysis of locomotor recovery following spinal cord injury using multiple, standardized gait speeds more fully characterizes that recovery.

P-38 **Pc-Based Cognitive Rehabilitation For Tbi: A Case Report**

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Traumatic brain injury (TBI) afflicts as many as 6 million Americans and is particularly prevalent among injured veterans returning from current conflicts in Iraq and Afghanistan. Many TBI survivors are faced with long-term disability and social isolation because of disabling cognitive impairments of attention, memory, and executive function. Here we describe the design and development of PC-based adaptive training procedures to improve cognitive outcome in TBI patients and report preliminary results of training from one case. **Case Report.** The 33 year old patient suffered a severe TBI (Glasgow Coma Score = 3) in a motor vehicle accident in 2003. Post-traumatic amnesia lasted 26 days. High-resolution MRI and diffusion tensor imaging (DTI) revealed widespread cortical thinning and significant damage to subcortical fiber tracts. Standardized neuropsychological tests (NPTs) revealed severe impairments of immediate and delayed memory, attention, and executive function. Performance was below the fifth percentile on most measures. Additional computerized tests showed perceptual, motor, and cognitive slowing. **Methods.** Adaptive, computerized training protocols were developed to train digit span, visuospatial span, and verbal memory. The computer training was performed in the laboratory for 1.5 hours/day for one week. A PC was installed in the patient's home, and computer training continued for three more weeks. The patient's performance was monitored by automated uploads of training data to our research site. **Results**. Performance improved on all of the training tasks. Post-training NPTs showed improvements of 5-30 percentile points with some scores reaching the low normal range. **Conclusions**. PC-based adaptive training is a promising tool for rehabilitating cognitive deficits in TBI.

P-39 **Cortical Processing Of Sensorimotor Information During Treadmill Locomotion In Adult Rats Spinalized As Neonates**

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The somatotopic map of the cortex changes after spinal cord injury. Furthermore, adult animals with neonatal spinal cord injuries exhibite greater changes in the somatotopic organization of the somatosensory cortex compared with animals that were injured as adults. Neonatally spinalized rats also have better functional outcome, suggesting a link between the changes in the somatotopic organization and functional recovery. However, since the majority of these studies addressed only the processing of passive sensory stimulations in the cortex when an animal is anesthetized, it is not known if the changes in the somatotopic organization are functionally relevant during processing of active sensorimotor stimulations in the awake, freely moving animal. To address this issue, we chronically implanted arrays of microwire electrodes into the infragranular layer of the hindlimb somatosensory cortex of adult rats with neonatal T8/T9 transections. The responses of single neurons were recorded in spinalized rats and normal adult rats during passive sensory stimulation and during active sensorimotor stimulation (treadmill induced locomotion) of the forelimbs in the awake, freely moving rat. Our results demonstrated that cortical neurons recorded from the spinalized rats were more likely to respond to both types of stimulations and responded with more spikes per stimulus, than cells recorded from normal rats. These results suggest that cells recorded from the infragranular hindlimb somatosensory cortices of adult rats spinalized as neonates are better able to encode stimuli applied to the forelimbs than cells recorded from normal adult rats. Therefore, the functional change in the somatotopic organization of the somatosensory cortex in response to passive sensory inputs after spinal cord injury extends to the processing of active sensorimotor inputs in the awake animal during treadmill induced locomotion.

P-40 **Shallow-Water Walking: Investigating A Novel Rehabilitation Strategy For Spinal Cord Injury**

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In an effort to better understand the contributions of limb-loading, cutaneous feedback and limb kinematics to a successful activity-based rehabilitation strategy for spinal cord injury (SCI), we are investigating two novel animal models. We recently showed that swim-training can bring about improvements in swimming following a moderately-severe contusion injury, but no improvements were seen in overground locomotion. The current study utilized shallow-water walking, where buoyancy provides some body weight support while still allowing for partial limb-loading and cutaneous afferent feedback during re-training. Our first goal was to determine how injured, but un-trained animals walk when placed in shallow water. Our second goal was to assess whether or not shallow-water walking can bring about improvements in locomotion in animals with incomplete SCIs. In the first study we assessed un-trained adult female SD rats with 25 and 50g-cm T9 contusion injuries at 1, 2 and 3 weeks post-injury by placing them in a plexiglass tank with or without 2" of water and using digital video to quantify paw-placement and hindlimb kinematics. In the second study, injured rats were group-trained (3-4 at a time) in 2" of water for 40minutes a day, 4 days a week for 6 weeks starting 1 week post-injury. Un-trained animals received equivalent amounts of group housing. We found that injured but un-trained animals show frequent coordinated plantar stepping when walking in shallow water as early as one week post-injury. However, when used as a re-training strategy, shallow-water walking did not bring about functional improvement beyond the normal course of locomotor recovery. These observations show that animals with severe, but incomplete thoracic SCIs are capable of generating quality plantar stepping when provided with partial body weight support and suggest that the normal course of functional recovery cannot be influenced by a re-training strategy based on partial body-weight support.

P-41 **Reduced Neural Degeneration And Improved Neurofunctional Outcome With Enriched Environment Combined Multimodal Early-Onset Stimulation (Meos) After Experimental Traumatic Brain Injury**

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To determine whether a paradigm of enriched environment (EE) combined with multimodal early onset stimulation (MEOS) after experimental traumatic brain injury (TBI) would reduce neural degeneration and to demonstrate behavioural correlates. Male Sprague-Dawley (SD) rats were subjected to lateral fluid percussion (LFP) brain injury or sham operation. Thereafter, half of the animals (injured and sham) were placed in standard housing (SH), the other half underwent EE+MEOS for a survival period of 15 days. EE consisted of serial cages with beddings, inclining platforms, and toys; MEOS comprised multimodal stimulation including auditory, visual, olfactory, and motor stimuli. Direct structural effects of traumatic brain injury were examined using Fluoro-Jade staining allowing identification of degenerating neural cell bodies and processes. Neuromotor performance and recovery was assessed prior to injury, 24h post-injury and on post-injury day (DPI) 15; cognitive function was assessed on DPI 11–15 using the Barnes circular maze (BCM). Statistical analysis revealed significantly lower numbers of Fluoro-Jade positive cells (degenerating neurons) within the penumbra of the brain lesion in the EE+MEOS group compared to the SH only group ($p < 0,005$) on DPI 15. EE plus MEOS animals also performed significantly versus SH only animals when tested for neuromotor recovery on DPI 15 ($p < 0,05$). Similarly, latencies to locate the hidden box under the BCM platform were significantly shortened in EE+MEOS animals on DPI 15 ($p= 0.003$). Exposure to EE+MEOS after TBI was associated with

reduced neural degeneration and improved neurofunctional outcome compared to SH only on DPI 15. This study was supported by the Koeln-Fortune Program, University of Cologne (Germany).

P-42 **Depletion Of Spinal 5-Ht Accelerates Mechanosensory Recovery In The Deafferented Spinal Cord**

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Brachial plexus avulsions in humans are severe neurological injuries causing disabling sensory and/or motor functional impairment in the affected limb. Such impairment is due to the permanent disconnection of nerve roots from the spinal cord. This has various consequences, including loss of normal sensation and paradoxically, the development of neuropathic pain. In rats, injury to a small number of cervical roots also results in mechanosensory deficits and neuropathic pain which have reproducible patterns of development and resolution; our lab has previously shown that dorsal root injury (DRI) of the C7 and C8 roots produces a deficit in low-threshold cutaneous mechanosensation in the ipsilateral forepaw, which spontaneously improves within 10 days, but does not completely recover to preoperative levels; the same injury results in the development of cold-pain in the ipsilateral forepaw which peaks at 10 days and recovers to pre-operative levels by 20 days post-injury. Further, this injury results in sprouting of serotonergic fibres descending from the brain. To further clarify the role of the descending serotonergic system in mechanosensation and pain following DRI, we investigated the effects of ablating serotonergic input to the spinal cord via intrathecal injection of 5,7 di-hydroxytryptamine (5,7-DHT) administered prior to C7/8 DRI. An immunohistochemical analysis of the cervical spinal cord 20 days post DRI assessed the efficacy of the toxin. Behavioural experiments revealed that 5,7-DHT treatment improved recovery of mechanosensation by approximately 6 days post injury, but had no effect on the development and resolution of cold pain. These results may provide better understanding into the mechanisms underlying mechanosensory dysfunction and pain following DRI.

P-43 **Task-Oriented Motor Training Promotes Recovery Of Grasp Function After Cervical Spinal Cord Injury**

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Cervical spinal cord injury (SCI) results in specific deficits in forelimb function. In the rat, lesions to the rubrospinal tract impair forelimb function despite the presence of an intact corticospinal tract, making it a behaviorally relevant pathway to study. Task-oriented practice is widely used in clinical neurorehabilitation to maximize recovery of function, but very few studies have been reported in the animal literature for cervical spinal cord injury. Eighteen adult female Sprague-Dawley rats with a preference to use their right forelimb to reach for single food pellets received a lesion to the right cervical dorsolateral funiculus. All animals received an injection of a liquid collagen matrix into the lesion site. Starting 5-7 days post-lesion, 8 animals were housed in a multilevel cage environment that promoted climbing and foraging for food (Motor Training group). Starting at 9 days post-lesion, the Motor Training group also spent 20-minutes/day, 5 days/week in a reaching trough apparatus that provided task-oriented practice by encouraging reaching by the impaired limb for food pellets. The control animals (n=10) remained in standard Plexiglas cages (Lesion Only group). All animals were assessed pre-injury and during weeks 1 and 8 postoperatively on two reach-to-grasp tests (Single Pellet and Staircase Reaching). The Motor Training group achieved significant recovery of function in the Staircase Reaching test at week 8 of recovery. This represented a highly functional improvement compared to the performance of the Lesion Only group. Group comparison on the single pellet test revealed no

differences in performance. Thus, task-oriented motor training improved reach-to-grasp performance in the staircase test, which was similar in design to the reaching trough apparatus used in daily motor training, but did not carryover to reaching performance that required greater accuracy (single pellet reaching). Supported in part by Grant# 2378 from the PVA Research Foundation, Stacy Anne Vitetta '82 professorship from Arcadia University, & P01 NS24707.

P-44 **Enhancing Grasp Control In Children With Spinal Cord Injury**

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We examined how children with tetraplegia due to spinal cord injury improve control of a grip-lift task, before and after surgical restoration of grasp and subsequent rehabilitation. Sixteen children with tetraplegia, 8 to 20 years of age, who underwent tendon transfers (TT) (N=8) or implant of the Freehand® (FH) system (N=8) participated. Each child grasped and vertically lifted, novel objects, equipped with 2 multi-axial force transducers, of different weight and texture, 10 times each. A magnetic, position sensor (Polhemus Fastrak, Colchester, VT), mounted on each object was used to track object position. Efficiency (time spent in phases of the grip-lift task) and anticipatory force scaling were measured during repeated object lifts over three time periods: 1) pre-operative baseline; 2) 2 months post-operative; and 3) 6 months post-operative. Sensibility, dexterity, strength and functional data were also collected at each period. The results indicate that strength and dexterity improved from baseline to post-operative periods for the TT (both, $p<0.05$) and FH (both, $p<0.05$) groups, while sensibility and overall function showed little change across period for both groups (all, $p>0.05$). Efficiency of task performance and anticipatory force scaling improved across trials within each period for objects of varied weight and texture (all, $p<0.05$) and between testing periods particularly among objects with different textures for the TT group ($p<0.05$). The findings suggest that surgical enhancement of motor output improved grasp control needed for object manipulation. With rehabilitation and practice post-operatively, both groups became more efficient at the grip-lift task and displayed effective anticipatory control strategies. However, the TT group, with greater sensibility, made the most improvement.
Acknowledgement: This study was supported by the Shriners Hospitals for Children.

P-45 **Recovery Of Forelimb Function After Cervical Spinal Cord Injury In Adult Rats**

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We investigated the effect of a rehabilitative strategy, enriched housing with daily forelimb training, on the recovery of skilled and unskilled forelimb function after a cervical spinal cord injury in adult rats. We examined the effects of acute and delayed intervention. Rats received a C4/C5 over-hemisection lesion which interrupts the right side of the spinal cord and interrupts the dorsal columns bilaterally. Post-injury, animals were divided into those housed in enriched environments with daily training in skilled reaching and gridwalk, versus those housed in standard cages without post-injury training (acute). A separate set of animals was housed for one month after injury in standard cages before separating into standard and enriched housing with daily training (delayed). Rolipram, a phosphodiesterase IV inhibitor, was administered to a subset of animals from both acute and delayed treatments to promote neuronal plasticity. Acutely treated animals were tested for forelimb function at 1,2,3 and 4 weeks after injury. Delayed treated animals were tested at weeks 1-6 after treatment was initiated. Enriched environments/daily training significantly improved skilled left forelimb function during reaching and improved sensorimotor function of the limbs on the gridwalk in both the acute and delayed treatment groups. Animals treated acutely with either daily training or rolipram preserved corticospinal tract fibers in the dorsal columns rostral to injury. There was no preservation of the corticospinal tract observed after delayed treatment. These results suggest enriched housing with daily forelimb training supports recovery of skilled forelimb use and promotes corticospinal plasticity in the absence of any

cellular replacement at the injury site. These studies suggest that both short and long term rehabilitation strategies contribute to neuroplasticity and functional recovery after spinal cord injury. Supported by NIH NINDS RO1s NS27054, NS051656 and the Christopher and Dana Reeve Foundation.

P-46 **Assessment Of Gripping Ability After Bilateral Cervical Contusions Of Increasing Forces In Mice**

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Recovery of arm/hand function can increase the quality-of-life in people living with a cervical spinal cord injury (SCI), motivating current efforts to develop cervical injury models and methods for functional assessment of forelimb function in mice and rats. Here, we describe a bilateral cervical contusion model in mice and assessment of forelimb impairment and recovery using a grip strength meter (GSM). Spinal cord injuries of differing severities were created at cervical level 5 (C5) using the Infinite Horizon Device by delivering forces ranging from 30-100 kDynes. Forepaw grip strength was assessed for 60 days post injury (DPI) using the GSM as described previously (Anderson et al., 2004). Mice that received 30 kDyne force injuries did not exhibit impairments in gripping ability. Mice that received 75 kDyne force injuries showed impairments in grip strength at early intervals after the injury, but recovered to about 85% of preoperative baseline control by 15 DPI. Mice that received 100 kDyne force injuries were unable to grip for 3 DPI then slowly recovered gripping ability to an average of 57% of baseline between 15-30 DPI. Although there was mortality during the surgical procedures to create the lesions, mortality was less than 10% in mice that survived the surgical procedure. Bladder function was minimally impaired even in the most severely injured groups. Although mice with 100 kDyne force injuries had very apparent disabilities within the first 10 DPI, they were able to maintain upright posture, move around in their home cages and feed independently by 1-3 DPI. Overall motor disability was not evident following lower force injuries. Our results indicate that bilateral cervical contusion injuries are feasible in mice and demonstrate that the GSM is useful for assessing forelimb function after cervical contusion injuries.

P-47 **TGF-α Infusion Increases Cell Proliferaiton, Alters Glial Scar Formation, And Enhances Axonal Growth Following Contusion Injury In Mice**

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Astrocytes play a complex role in the cellular response to trauma in the central nervous system. Strategies that seek to improve endogenous repair mechanisms must successfully tip the balance toward the beneficial roles of astrocytes in neuroprotection and axonal guidance and limit the role of these cells in establishing barriers to axonal growth and neural plasticity. Transforming growth factor-α (TGF-α) is a ubiquitous factor that is present in the CNS, active on astrocytes both *in vitro* and *in vivo*, and is poised to contribute to such a repair strategy, yet its role in repair after injury is understudied. To determine if exogenous TGF-α can be used to promote the beneficial effects of astrocyte activation, human recombinant TGF- was administered intrathecally for two weeks immediately following moderate contusion injury to the midthoracic spinal cord in adult female C57BL/6 mice. The animals survived for 21 days after injury, and effects of treatment on cell proliferation, glial activation, functional recovery, and axonal growth were assessed. TGF-α infusion enhanced cell proliferation during the first week after injury and altered the composition of the lesion site, particularly in the caudal-most regions. The lesion site was characterized by markedly increased GFAP and neurofilament immunoreactivity in animals treated with TGF-α compared to vehicle. This suggests that TGF-α infusion can alter the lesion environment to make it more permissive for both astrocyte invasion and axonal growth after spinal cord injury. Supported by NS043246, NS045758.

P-48 **The Inhibitory Influence Of Glial Scar On Cell Therapeutic Strategies After Chronic Spinal Cord Injury**

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While cell-based therapies for the repair of SCI show the greatest success in the acute or subacute stage, less attention has been given to facilitate repair of the chronically injured spinal cord mainly due to the complexity of chronic SCI pathobiology and the inherent challenges.

Recently our team has shown that a cellular therapy using adult neural precursor cells (NPCs) when combined with growth factors and the neuroprotective/anti-inflammatory drug minocycline promotes significant remyelination and improves neurological function after subacute contusive SCI (2 weeks post-injury). However, our observations suggest that the therapeutic efficacy of NPC transplants in chronic SCI is negatively influenced by chondroitin sulfate proteoglycans (CSPGs) in astrocytic scar. CSPGs are key components of glial scar, which significantly contribute to the abnormal modifications of ECM and potently impede axonal regeneration and sprouting. Enzymatic digestion of CSPGs enhances neural plasticity associated with neurological recovery after SCI. We **hypothesized** that a combined therapeutic strategy including 1) transplants of NPCs, and 2) approaches to modify the inhibitory properties of CSPGs in the glial scar will significantly improve the outcome of cell-therapeutic approaches using adult NPCs in chronically injured spinal cord. We aimed to: **1)** pharmacologically target the CSPGs using chondroitinase ABC (ChABC) prior to NPC transplantation and **2)** examine the effects of these interventions on the survival, migration, integration and remyelination abilities of adult NPCs after chronic transplantation. We show that treatment with ChABC at 5-7 weeks post SCI significantly reduced the CSPG deposits in the chronically injured spinal cord and induced the appearance of degraded GAG side chains products. Furthermore, application of this approach prior to NPC transplantation substantially increased the long-term survival of NPCs in chronically injured spinal cord. **Conclusions:** We propose that approaches to attenuate the inhibitory properties of glial scar would further facilitate the NPC-mediated repair of chronic SCI.

P-49 **Cell Permeant Peptidomimetics That Manipulate Sensory Neuron Cytoskeletal Components May Facilitate Regeneration Through Glial Scar Chondroitin Sulfate Proteoglycans**

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A major impediment to regeneration is the formation of a glial scar that expresses axon growth–inhibiting chondroitin sulfate proteoglycans (CSPGs). Technologies that could overcome the inhibitory effects of CSPGs could lead to major advancements in therapies to promote functional recovery after CNS injury. Toward this goal, we are using cell-permeant peptidomimetics (CPP), a novel technology that directly manipulates protein activities in cells. CPP technology uses short peptide sequences to mimic specific protein motifs or regions. When introduced into cells, these peptides interfere with specific protein activities, and alter/regulate growth cone function. When fused to other peptides, proteins, and nucleic acids, CPPs facilitate translocation of the fusion partner into cells. The mechanism of translocation is not clearly understood, but charge, hydrophobic content, and/or secondary structure are deemed critical factors. We are targeting cofilin, a cytoskeletal protein that regulates filopodial turnover and is implicated in the inhibitory growth cone responses to CSPGs, such as stalling and turning. *In vitro*, fluorescence microscopy assays were used to select an optimal peptide transport system for embryonic chick and rat dorsal root ganglion (DRG) sensory neurons, and biochemical assays were used to screen cofilin-related peptides (CRPs) designed to manipulate cofilin protein activities. Growth cone motility assays were used to assess the efficacy of the selected peptide transport system and CRPs for altering

cofilin activity in a manner that promotes axon growth across CSPG adsorbed substrata. This on-going study is revealing the strengths and issues related to using this technology to promote axonal regeneration. Support: NIH SBIR NS051878 (MTW, MM, DMS).

P-50 **Immune-Mediated Wound Healing Acts With NT-3 To Support Axonal Sprouting After Spinal Cord Injury**

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After a unilateral corticospinal tract lesion (CSTL) at the level of the medulla over-expression of Neurotrophin-3 (NT-3) induces axonal sprouting of the intact CST in the acutely injured but not uninjured or chronically injured spinal cord. Since a CSTL induces Wallerian degeneration (WD) and activates microglia we propose that processes associated with immune-mediated wound healing participate to induce neuroplasticity. To test if an immune response mediates axonal sprouting we measured the effect of NT-3 expression on sprouting in rats immunosuppressed with antibodies to the CD4 and CD45 receptors. NT-3 was over-expressed 2 wk later in the lumbar cord with an adenoviral vector carrying the NT-3 gene targeted to spinal motoneurons by retrograde transport. At 35d post-lesion we measured the number of CST axons that traversed the midline to the source of NT-3. No axonal sprouting was induced in immunosuppressed rats compared to controls. We tested whether re-evoking an immune response in chronically lesioned rats would induce plasticity. Rats with chronic (=4m) CSTLs were treated with systemic injections of lipopolysaccharide (LPS) 7d before NT-3 was over-expressed. LPS re-activates immune response associated with WD. Axonal sprouting was greater in the rats treated with LPS compared to controls. Activation of microglia and macrophages that function as phagocytes was increased in rats treated with LPS compared to controls. However, immunosuppression did not decrease the number of phagocytic activated microglia. These results demonstrate that processes associated with immune-mediated wound healing play a role in mediating NT-3-induced neuroplasticity after injury. Supported by grants from NIH, Christopher and Dana Reeve Foundation, and Mission Connect of the TIRR Foundation.

P-51 **Using Designer Pgs To Reveal Inhibitory Motifs Of Glial Scar Chondroitin Sulfate Proteoglycans *In Vitro* And *In Vivo*.**

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Spinal cord injury (SCI) is a debilitating condition afflicting approximately 250,000 people in the U.S. alone. This condition presents formidable challenges to successful post-trauma recovery of function and often results in permanent CNS damage. Chondroitin sulfate proteoglycans (CSPGs), extracellular matrix molecules expressed by astrocytes of glial scar tissue, inhibit regeneration following SCI. PGs are large, aggregating macromolecules with multiple complex structural units. Although it is known that the glycosaminoglycan (GAG; sugar) moieties inhibit axonal regeneration, the specific structural elements of the GAG chains responsible for inhibition remain unidentified. In addition, the effects of such structural moieties on neuronal growth cone behaviors have yet to be quantified. In an on-going study, we aim to systematically identify inhibitory CSPG microheterogeneities using a novel array of CSPGs referred to as “Designer PGs,” which contain engineered modifications in the GAG chains and/or the protein core of the neural CSPG, aggrecan. Using image analysis, we qualitatively and quantitatively gauge changes in growth cone behaviors and morphology in response to these Designer PGs, using novel and established bioassays *in vitro*. This data is then organized according to behavior type and used to assign an “inhibitory quotient”, or IQ, score to a given Designer PG. Through this gradual identification of the

precise structural moieties responsible for CSPG-induced inhibition, future clinical therapies will be able to selectively target the most inhibitory domains of glial scar CSPGs to facilitate regeneration and restoration of function following SCI, while leaving the repair-required motifs intact.

P-52 **Decorin Promotes Robust Axon Growth On Inhibitory Cspgs And Myelin Via Direct Effect On Neurons**

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Axon growth inhibitory chondroitin sulfate proteoglycans (CSPGs) and myelin associated molecules are widely accepted as major impediments to axon regeneration within the injured adult central nervous system (CNS). Two potential means of overcoming the effects of these inhibitors are to lower their levels within the injured CNS or to block neuronal sensitivity to these molecules. We have demonstrated that spinal infusion of the small leucine rich proteoglycan, decorin can suppress the levels of multiple scar associated inhibitory CSPGs, an effect that likely contributed to the ability of axons to cross decorin treated spinal cord injuries (Davies et al., Eur.J. Neurosci., 2004). However in light of data from Koprivica et al., (Science, 2005) that showed that blocking epidermal growth factor receptor (EGFR) activity on neurons promoted neurite extension on both inhibitory CSPGs and myelin, there remained the possibility of a direct effect of decorin on the ability of neurons to grow on these inhibitors via decorin inhibition of EGFR activity (Csordas et al., J. Biol. Chem., 2000). We have therefore conducted an *in vitro* analysis of the effects of decorin on neurite extension by adult dorsal root ganglion (DRG) neurons grown on substrates of inhibitory CSPGs or myelin membranes mixed with laminin. Decorin treated DRG neurons grown on a CSPG / laminin substrate for 48hrs showed a ~14.5 fold increase in average neurite length per neuron compared to untreated controls (av. 218µm +decorin: av. 15µm untreated). Decorin treated DRG neurons grown on a myelin / laminin substrate for 18hrs showed a ~4.8 fold increase in average neurite length per neuron compared to untreated controls (av. 160µm +decorin: av. 33µm untreated). Decorin therefore represents a novel combination therapy for promoting axon regeneration via suppression of inhibitory scar tissue formation and directly boosting the ability of axons to grow within CSPG or myelin rich tissues of the injured CNS.

P-53 **Inhibition Of Necrosis With The Calpain Inhibitor Mdl28170 Enhances The Survival Of Schwann Cells Transplanted Into The Injured Adult Rat Spinal Cord**

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Despite the diversity of cells available for transplantation into spinal cord injuries (SCIs), functional improvement associated with cellular transplantation has been limited. One factor limiting the efficacy of transplanted cells is their poor survival. Recently we demonstrated a rapid, early loss of Schwann cells (SCs) within the first 24 hours after transplantation; with fewer than 20% of cells surviving beyond one week when implanted into a one-week old spinal cord contusion. Furthermore, we showed that transplanted SCs die by necrosis and apoptosis, with necrosis predominating. In the current study we tested whether pretreatment of SCs with inhibitors of necrosis and/or apoptosis could enhance the SC survival 7 days after transplantation. Factors present after SCI at the injury site have been implicated in tissue necrosis, including elevated lipid peroxidase and iNOS activity and activation of calpains. In vitro, inhibitors of either lipid peroxidation or calpain activation increase SC survival following induction of necrosis. And inhibitors of caspase activity prevent SC apoptosis. In the current study we tested whether application of the lipid peroxidase inhibitor, U83836E, the iNOS inhibitor, SB203580, or the calpain

inhibitor, MDL28170, to cells prior to and at the time of transplantation could reduce transplanted cell loss associated with necrosis; and whether treatment with the caspase inhibitors YVAD or ZVAD could prevent SC death associated with apoptosis. Blocking calpain activation doubled the number of surviving SCs. Transplanted SCs treated with the caspase inhibitor YVAD showed a trend towards improved survival. Together this suggests that multiple factors present within the injury environment contribute to the death of transplanted cells, and that strategies aimed at improving the survival of transplanted cells need to address the multiple causes of necrosis and apoptosis present within the injury environment. Supported by: Helen Wilshire Walsh fund, The State of Florida, The Miami Project and NINDS 09923.

P-54 **Astroglial Nogo-A Expression After Stroke**

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We have previously shown that anti-Nogo-A immunotherapy enhances functional recovery and neuronal axonal and dendritic remodeling after stroke. To further understand the cellular distribution of Nogo-A after stroke and define possible targets for our immunotherapy, we performed anti-GFAP and anti-Nogo-A immunohistochemistry in normal rats and at 1, 3, 7, 14, and 28 days after permanent middle cerebral artery occlusion (MCAO). GFAP-positive astrocytes were observed in perilesional cortex and white matter structures including the ipsilesional external capsule and corpus callosum at all time points after stroke. A subset of the GFAP-positive astrocytes found in post-stroke brains were also positive for Nogo-A. Double-positive cells were present at all time points examined, but were most abundant at 3 days after stroke. We extended our *in vivo* findings by exposing cultured astrocytes to hypoxic conditions *in vitro*. Nogo-A immunopositivity on cultured astrocytes increased over time to 72 hours post-hypoxia, in agreement with the post-stroke findings. These results have been extended via Western blot analyses, which confirm the post-hypoxia increase in astroglial Nogo-A expression *in vitro*. However, the cellular role of astroglial Nogo-A and its relative importance as an inhibitor of functional recovery and neuroanatomical remodeling remain unknown. Supported By: NINDS 40960, Department of Veterans Affairs, the Loyola Neuroscience Institute, Novartis, and the Swiss NSF.

P-55 **Galectin-1 Facilitates Macrophage Accumulation In Intact And Injured Peripheral Nerves**

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Although axons in the peripheral nervous system have the ability to regrow following injury, their regenerative success is often poor. Efficient degeneration of debris in the injured nerve is necessary for proper peripheral axonal regeneration and is characterized by activation and migration of Schwann cells and infiltration and activation of hematogenous macrophages. Activated macrophages generate myriad cytokines and are especially important in the phagocytosis of inhibitory myelin and axonal debris; thus, these cells are critical in the production of an environment permissive to axonal outgrowth and in the mediation of a robust regenerative response. Numerous cytokines have been implicated in the recruitment/activation of macrophages following peripheral nerve injury. Galectin-1 (Gal1) is a protein that may have cytokine-like effects on macrophages: macrophages exposed to Gal1 express and release an axonal regeneration-promoting factor. Moreover, Gal1 expression in neurons is correlated positively with regenerative potential. Using in vivo models, we have found that Gal1 is involved in the

accumulation of macrophages. We demonstrate that exogenous Gal1 is sufficient to facilitate accumulation of macrophages in the uninjured peripheral nerve, and that Gal1 is required for proper macrophage recruitment to various peripheral nervous tissues following sciatic nerve ligation. Gal1 null mutant (*Lgals1*-/-) mice showed impaired injury-induced recruitment of macrophages to areas associated anatomically with sciatic nerve ligation (the distal sciatic nerve and L5 dorsal root ganglion). Injection of Gal1 into nerves of *Lgals1*-/- mice was not sufficient to promote accumulation of macrophages, and macrophage responses were impaired following peripheral nerve transplant, suggesting that cytokine cascades are altered in these mice. We will also establish whether injury-induced neutrophil accumulation and axonal degeneration are altered in *Lgals1*-/- mice. Our data implicate Gal1 in the recruitment of macrophages to the nervous system following peripheral nerve injury and indicate that this protein may affect macrophage cytokinesis.

P-56 **Macrophage-Mediated Regeneration And Neuropathology In The Adult Central Nervous System**

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Under normal circumstances, neurons of the central nervous system (CNS) fail to regenerate after axotomy. Although CNS neurons retain the ability to regenerate after development, finding stimuli to encourage intrinsic regrowth remains elusive. Activated macrophages may provide this stimulus; the presence of these cells in the eye and dorsal root ganglion (DRG) induces regeneration of transected axons. However, the pro-regenerative macrophage activator, zymosan, causes cell loss and tissue pathology when injected into the CNS. The dichotomous nature of zymosan-activated macrophages is rarely studied simultaneously. The current work investigates the neurodestructive and regenerative potential of this powerful macrophage activator. In vitro, axon growth and sprouting increased 1.5 times for DRG neurons grown in zymosan-activated vs. unstimulated macrophage conditioned media (MCM), however DRG cell survival was reduced by 81% in zymosan vs. unstimulated MCM. To address the dichotomous role of macrophages in vivo, we combined a well-established DRG microtransplantation technique (to assess regeneration; Davies et al., 1999) with intraspinal zymosan microinjection. The presence of activated macrophages 4mm from the DRG transplant modestly increased the average distance of axon growth from the DRG transplants (4.62 +/- 0.39mm) compared to controls (3.21 +/- 0.49mm; P=0.057). Axonal growth, however, was reduced when macrophages were activated in close proximity (2mm) to the DRG transplant (average growth = 2.98 +/- 0.37mm; p=0.018). Intraspinal zymosan injections induced cavitation and tissue pathology. Macrophages mediated this neurodestruction as depletion of peripheral macrophages reduced the lesion area by 47% compared to non-depleted controls (p=0.033). Our findings suggest that recruitment of activated macrophages to the intact spinal cord can induce a modest, proximity-dependent, axonal regeneration. However, this recruitment is associated with dramatic cell loss and tissue cavitation. The search continues for a means of activating macrophages in such a way that the scales can be tipped toward an overall beneficial response.

P-57 **Increased CNTF And FGF-2 Expression In Regions Of Elevated Oligodendrocyte Genesis After Spinal Contusion**

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Oligodendrocyte (OL) loss following spinal cord injury (SCI) is well documented. Recently, we showed that oligodendrocyte progenitor cell (OPC) accumulation and robust oligodendrogenesis occur along spinal contusion lesions in the first 2 weeks post-injury. Since ciliary neurotrophic factor (CNTF), an astrocyte-derived growth factor, promotes OPC proliferation and differentiation into OLs and is

upregulated in several CNS disorders, we hypothesized that CNTF expression is increased after SCI, especially in the regions of enhanced oligogenesis. Using a rat spinal contusion model, we quantified CNTF protein after SCI using Western blots. This revealed that CNTF expression continually rises between 5d and 28d post-injury (dpi). Using immunohistochemistry, we next examined tissue sections spanning the lesions at 3-28 dpi to determine the spatiotemporal distribution of CNTF. CNTF was significantly increased in spared WM and GM at 5, 7, 14 and 28dpi compared to uninjured controls; CNTF was not expressed in lesion cavities. At 7dpi, CNTF was equally high along lesion borders and outer spared tissue; by 28dpi CNTF was significantly elevated along lesion borders compared to spared tissue. CNTF can potentiate fibroblast growth factor-2 (FGF-2) expression; hence we quantified FGF-2+ve cells in the same regions as above. Indeed, significantly higher FGF-2+ve cells were noted along lesion borders and in spared GM suggesting that CNTF may promote FGF-2 expression in these regions. Since CNTF is upregulated in regions of prominent oligogenesis, CNTF may play a direct and/or indirect role in OL replacement after SCI. Collectively, these data elucidate a potential previously un-appreciated mechanism for CNS endogenous repair.

P-58 **Bdnf Governs The Plasticity Of Nociceptive Circuitry Following Spinal Deafferentation**

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Dorsal root injury (DRI) results in spinal deafferentation and permanent sensory dysfunction, such as loss of mechanosensation and enhanced pain. Modifications to sensory transmission and processing in the spinal cord which elicit such consequences may involve the strengthening of existing synapses, the unmasking of silent synapses or the establishment of new connections subsequent to axonal sprouting. Brain-derived neurotrophic factor (BDNF) is upregulated in the spinal cord following DRI and has multiple effects on TrkB-expressing neurons, including potentiation of nociceptive transmission and stimulation of axon outgrowth. We therefore investigated the role of endogenous BDNF on the plasticity of pain circuitry in the deafferented dorsal horn. Transection of the C7 and C8 dorsal roots (C7/8 DRI) induced rapid (3 days post-DRI) and persistent (20 days) upregulation of BDNF by microglia, and elicited the sprouting of TrkB-expressing GABAergic interneuronal processes in the dorsal horn. Sequestration of endogenous BDNF via intrathecal TrkB-Fc infusion not only attenuated GABAergic system plasticity, but also promoted nociceptor terminal sprouting. C7/8 DRI also generates cold pain in the denervated forepaw, which peaked at 10 days and resolved by 20 days post-injury. Continuous TrkB-Fc treatment prevented this recovery illustrating that BDNF also contributes to cold pain resolution. These findings demonstrate opposing influences of BDNF on the plasticity of pain-transmitting and –modulating circuitry in the dorsal horn, which are likely to underlie cold pain behaviour following C7/8 DRI.

P-59 **Cellular Replacement Modulates Endogenous Respiratory Behavioral Neuroplasticity Following High Cervical Spinal Cord Injury**

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Interfacing cellular and molecular interventions with endogenous neuroplasticity represents a major objective of spinal cord repair. In the present study, we tested whether a cellular replacement strategy would modulate recovery of ventilation after high cervical spinal cord injury. For these experiments, E_{14} rat spinal cord tissue was grafted either as whole pieces (FSC_W) or as microdissected floor- (FSC_V) or roof-plate (FSC_D) regions into acute C_2 hemisections of the adult rat spinal cord. Thereafter, barometric plethysmography was employed at routine intervals to assess the pattern of ventilation. This analysis revealed no group differences until 8 weeks post-transplantation at which time

FSC_V graft recipients (n = 9) exhibited a lower frequency of breaths (P<0.01, one way ANOVA) than seen either in FSC_D (n =9) or whole FSC graft recipients (n = 6) under quiet room air breathing conditions. Further, analysis of normalized tidal volume (TV) showed a strong trend towards FSC_V graft recipients having larger TVs, falling slightly below significance (P=0.061, one way ANOVA). However, the TV for FSC_V recipients was significantly larger when directly compared to FSC_D recipients (P=0.036, t-test). As expected, no significant change in minute ventilation was observed. In other studies, we have found that FSC_V grafts consist of cells primarily resembling intermediate gray interneurons, whereas FSC_D grafts are comprised of matured substantia gelatinosa–like regions which are minimal at most in FSC_V transplants. In that context, the present findings reflect cellular specificity in promoting differences in certain components of respiratory behavioral recovery in this neuroplasticity model. The time-course of change in breathing pattern suggests that local spinal cord circuitry remodeling may be involved. Future neuroanatomical studies will address this issue directly.
Supported by NIH (5RO1NS54025).

P-60 **Plasticity In An Intersegmental Pain Reflex Following Spinal Cord Lateral Hemisection In The Rat**

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Previously, we reported the effect of spinal cord injury severity and recovery time on function in an intersegmental pain reflex whose neural circuitry spans the level of injury, the cutaneus trunci muscle (CTM) reflex. The CTM reflex produces a skin “shrug” in response to pinch on a rat's back and is mediated by a 3 neuron circuit: C and A-delta afferents in segmental dorsal cutaneous nerves (DCNs), ascending propriospinal interneurons, and the CTM motoneuron pool. The reflex can be quantified from the CTM muscle nerve neurogram in response to stimulation of the DCNs. The reflex is bilateral but asymmetric such that a larger reflex response is seen ipsilaterally than contralaterally. In this study, we evaluated the CTM reflex 1 week following a lateral hemisection on either the left or the right at T9 in Long Evans rats by sequentially stimulating the left and then right DCNs at L1, T12, T10, T8 and T6 and recording the left CTM neurogram. The animals’ spinal cords were then harvested for histological measurements.
In uninjured controls, the symmetry of the CTM response was best at mid to lower thoracic levels (T8, T10) and less above and below those levels (T6, T12, L1). The effect of hemisection on the CTM reflex ranged from it being abolished bilaterally below the injury level to it being at least partially preserved bilaterally there. Anatomical evaluation of the hemisection injuries failed to reveal a pattern of injury that could easily explain the range of physiological outcomes. In several cases where the CTM reflex showed bilateral preservation below the level of injury, the symmetry of the CTM responses between stimulating the right and the left T12 or L1 DCNs was greater than in controls, indicating that some infra-injury plasticity in the reflex can occur within a week of injury.

P-61 **Bone Marrow Stromal Cell Transplantation Into Spinal Cord Injury: Promises And Obstacles**

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Transplantation of bone marrow stromal cells (MSC) offers an exciting prospect for treatment of spinal cord injury (SCI) because of the ease of obtaining and culturing MSC and the potential for autologous transplantation. Among the issues that need to be resolved are donor variations, optimal cell profiles, effective culture conditions, and minimally invasive delivery methods. Furthermore, because MSC grafts only modestly improve function in SCI models, it is likely that combinatorial therapies will be needed to increase transplant efficacy. Our studies with human MSC have shown donor variations in secretion profiles and support of axon outgrowth, which could potentially influence the efficacy of cell

therapy, complicate the use of autologous transplants, and may require pre-selection of donors or clones. We are currently investigating the optimal donor cell profile with respect to therapeutic factor secretion, extracellular matrix production and surface molecule expression in correlation with efficacy in vivo. Optimal and well-defined cell culture conditions are crucial to allow rapid cell expansion (assuming autologous grafting) and to minimize augmentation of existing variations, including changes in MSC differentiation potential. We have optimized protocols to permit efficient cell expansion, and in parallel have analyzed changes in response to culture conditions. Establishing minimally invasive delivery methods will be extremely beneficial for clinical applications. In multiple studies, we have demonstrated that lumbar puncture is an efficient, non-invasive way to deliver cellular transplants. This procedure is relatively safe and unlikely to further risk SCI patients. We found that significant numbers of MSC enter hemisection and contusion injuries resulting in neural tissue protection. To further improve efficacy of MSC transplants, we are currently investigating combinatorial therapies, including the use of neuroprotective drugs, neurotrophin gradients and exercise paradigms. We have now also begun to compare MSC derived from bone marrow with those derived from umbilical cord blood.

P-62 **The Nucleoside Disphosphate Kinase, Nm23h1, Is Expressed By Human Bone Marrow Stromal Cells And Stimulates And Directs Neurite Outgrowth In Vitro**

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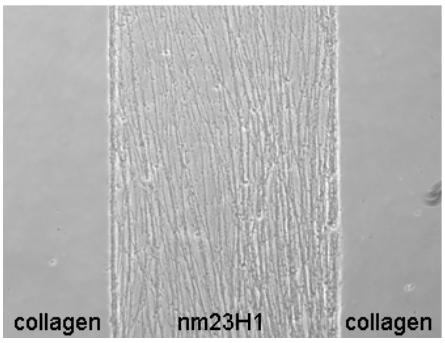
Transplantation of bone marrow stromal cells (MSC) in animal models of spinal cord injury encourages functional recovery. Recently we demonstrated that human MSC promote neurite outgrowth over CSPG, MAG and Nogo-A via direct contact-mediated events, but they also secrete neurotrophic factors. We have identified that MSC express the nucleoside disphosphate kinase, *nm23H1*. Previous studies have shown (i) transfection of PC12 cells with nm23M1 (the murine homologue) stimulates neurite outgrowth, depending on its kinase activity, (ii) cytosolic nm23H1 signals through the rho kinase family. However, nm23H1 has been demonstrated to exert extracellular effects, promoting cell survival. We found that MSC-conditioned culture medium is positive for nm23H1 by dot blot. We therefore examined the influence of extracellular nm23H1 on neurite outgrowth. Recombinant nm23H1 was adsorbed to nitrocellulose-coated culture plates in strips, which were then further coated with type I collagen. DRG explants were seeded onto these substrate “choice assays” and neurite outgrowth stimulated by NGF. When DRG explants settled onto collagen, neurite outgrowth advanced over the collagen with some degree of fasciculation, but as the neurites encountered nm23H1, they de-fasciculated and branched to form extensive neural networks. Neurites that subsequently encountered nm23H1: collagen borders turned and extended along the boundary, on the side of the nm23H1. Some growth cones collapsed at the nm23H1: collagen boundary. These effects were not seen with collagen: laminin choice assays. Hence, substrate-bound nm23H1 both promotes and directs neurite outgrowth. The effects were dose-dependent and did not occur when nm23H1 was in solution. Where DRG explants settled directly onto high concentrations of substrate-bound nm23H1, neurite outgrowth was extensive, rapid and limited to the nm23H1 (see above). Ongoing experiments are determining the signalling processes involved in how extracellular nm23H1 stimulates/ directs nerve growth and whether these effects are involved in the capacity of MSC to promote neural repair.



P-63 **Peripheral Delivery Of Adult Human Bone Marrow-Derived Stem Cells Following Ischemic Stroke Leads To Partial Functional Recovery And Cortico-Efferent Plasticity In Adult Rats**

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Ischemic stroke, resulting from lack of blood flow to the brain, leaves survivors with permanent neurological deficits for which no effective cure exists. A novel experimental approach that has tremendous potential to improve functional recovery after stroke includes stem cell transplantation. There is a need for evaluation of different routes of administration for more clinically amenable delivery. The purpose of the present study was to determine the efficacy of the route of administration of human adult bone marrow-derived stem cells (hABM-SCs) in a rodent stroke model by studying their functional recovery and neuroanatomical plasticity. Adult rats were trained on the skilled forelimb reaching task, and then underwent permanent middle cerebral artery occlusion. One week later, rats received treatment with hABM-Scs via intravenous delivery or intracranial delivery, or vehicle control. Animals treated with hABM-SCs transplanted intracranially or intravenously improved significantly as compared to vehicle controls in the forelimb reaching task. In addition, animals treated with hABM-SC delivered by both routes had significantly more cortico-efferent axonal plasticity originating from the intact, contralesional hemisphere as analyzed in the corticorubral and corticospinal pathways compared to stroke only vehicle control animals. Enhancement of neuroplasticity from uninjured brain areas is one mechanism by which human adult bone marrow-derived stem cell treatment after stroke leads to functional recovery.

P-64 **Neurorestorative Effects Of Autologous Transplants Of Adipose-Derived Adult Stromal (Adas) Cells In A Rat Spinal Cord Injury Model**

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In order to accomplish neural repair in SCI, several well recognized hurdles need to be overcome. Following spinal contusion injury there is tissue death at the injury epicenter leaving a empty cyst or post-traumatic syrinx that is inhospitable to axonal regeneration. This area is surrounded by a glial and extracellular matrix scar that is also inhibitory to regenerating axons.

Cell transplantation has been used in SCI as a neural repair strategy with a range of goals from neural tissue replacement to providing a tissue bridge to promote axonal growth across the level of injury and permit possible reconnection with their targets. Ideal cell transplant sources would possess several properties, namely autology (non-immunogenic), easily harvested in large numbers, non-tumorigenic and possibly safely transducible. A variety of cell types have been tried in SCI including embryonic stem cells, Schwann cells, olfactory ensheathing glia, bone marrow stem cells and skin derived progenitor cells but all of these have been met with limited success and we have only begun to scratch the surface of understanding how they cause any therapeutic benefit.

We present evidence here that transplantation of autologous adipose derived adult stromal (ADAS) cells into the site of a spinal cord contusion can 1) ameliorate the formation of the post traumatic syrinx and 2) at least partially overcome the growth inhibitory environment of SCI to promote aligned neurite outgrowth without causing tumor formation. Furthermore, we provide evidence for mechanisms by which ADAS transplantation could be effecting this neural repair process including production of neurotrophic substances, modulation of extracellular matrix composition, and modulation of chronic inflammation processes. These preliminary studies point to the potential of ADAS cell transplantation in neural repair and begin to dissect out mechanisms by which this cell transplantation therapy causes its beneficial effects.

P-65 **In Vitro Characterization Of Rat Adipose-Derived Adult Stromal (Adas) Cells And Neuroprotective Effects Of Autologous Adas Transplantation In A Rat Model Of Parkinson's Disease**

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Adult tissues of mesenchymal origin have been reported to contain progenitor cells with neurogenic potential. However, it is not clear whether the phenotypes these cells adopt *in vitro* are stable or whether their differentiation status is required for their reported ability to provide functional benefits in models of neurological injury or disease. We describe a population of adipose-derived adult stromal (ADAS) cells which can be expanded for several passages *in vitro*. Mitogen-withdrawal and exposure to serum-containing Neural Differentiation Medium (NDM) or to N2/valproate-supplemented serum-free medium induced extensive process outgrowth in ADAS cells, downregulation of nestin, and expression of early neuronal fate markers including NeuroD. The phenotypic stability after differentiation and re-exposure to serum was examined *in vitro* and found to vary between markers and to be dependent on the strength and duration of the stimulus. Exit from the cell cycle was achieved only after exposure to retinoic acid and forskolin.

To investigate the extent to which ADAS cell plasticity determined the ability of ADAS grafts to neuroprotect or aid in restoration of function in a lesioned dopaminergic pathway, i*n vitro*-expanded naïve and NDM-differentiated ADAS cells prelabeled with MitoTracker Red were autologously transplanted into substantia nigra 1-week after 6-hydroxydopamine intrastriatal injection. Although ADAS cells did not adopt dopaminergic cell fates *in situ,* neurochemical and behavioral measures confirmed the neuroprotective effects of ADAS grafts on dopamine neurons against oxidative neurotoxin-induced death. This study is the first to demonstrate that independent of the pre-transplantation differentiation status of the cells, autologous ADAS cell transplants exert neuroprotective effects in an oxidative neurotoxin model of Parkinson's disease.

P-66 **Forming A Neuronal Relay In The Injured Spinal Cord: Reconnecting The Sensory System Using Neural Stem Cells**

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The therapeutic promise of neural stem cell transplants in treating CNS injury and neurological disorders includes the exciting potential for cell replacement. As a proof of principle we focused our studies on grafting experiments that will test the ability of neural stem cells to form relays and reconnect interrupted sensory axons in the dorsal columns. The critical steps in forming neuronal relays include the identification of appropriate cells that will survive in the injury site, generate excitatory neurons, integrate with host spinal cord through synaptic connections, and connect with appropriate targets in the dorsal column nuclei. Our previous reports have shown that neuronal and glial restricted precursors (NRPs and GRPs, respectively), isolated from transgenic alkaline phosphatase (AP)-expressing rats, can be used for reliable fate analysis. NRPs grafted into the intact adult CNS survive, differentiate into neurons, and form structural synapses with the host. However, NRP require co-transplantation of GRPs for neuronal differentiation and survival in the injured CNS. We reasoned that GRPs generate supportive astrocytes and produce a micro-environment conducive to NRP survival and differentiation. In this study we have begun examining how mixed NRP/GRP grafts can be used for circuit reconstruction in the injured dorsal columns. First, we have shown that our grafts are capable of producing glutamatergic neurons necessary for an excitatory relay. Second, we explored the use of different matrices and growth factors to induce connectivity by showing that traced host axons can grow into the graft and express synaptic markers, suggesting synaptic connections with transplant-derived neurons. Third, we employed a BDNF-lentivirus vector (developed by Dr. Blesch) to establish a neurotrophin gradient and promote graft-derived

neurons to extend long axons towards their target, the dorsal column nuclei. These findings represent initial milestones in the challenging task of constructing a functional relay.

P-67 **The Neurogenic Basic Helix-Loop-Helix Transcription Factor Neurod6 Mediates Neuritogenesis And Neurite Regeneration Of Mouse Embryonic Stem Cells And Neural Precursor Pc12 Cells**

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During corticogenesis, NeuroD6 expression coincides with cell cycle exit and onset of terminal differentiation and is detected in dividing progenitor cells located in the subventricular zone that have not yet exited the cell cycle but are committed to become upper motor pyramidal neurons. Since our previous work revealed that NeuroD6 is a key regulator of the NGF pathway, we investigated whether NeuroD6 expression is associated with NGF-responsive sensory networks during spinal cord development. We found that NeuroD6 expression is restricted to the dorsal half of the neural tube and spinal ganglia. NeuroD6 expression was first detected in immature neurons located outside the ventricular zone before expanding into the mantle layer in the directions of the dorsal rootlets at later stages. To elucidate NeuroD6 function, we overexpressed NeuroD6 in two distinct cell models, neuronal progenitor rat PC12 pheochromocytoma cells and undifferentiated mouse CCE embryonic stem (mES) cells. Constitutive expression of NeuroD6 led to spontaneous neuritogenesis and differentiation of PC12 cells by favoring a distinct combination of gene products, many of them being critical building blocks for neuritogenesis and regeneration. NeuroD6 also promoted neurite regeneration, a process that was further enhanced upon cAMP exposure. More importantly, NeuroD6 expression allowed PC12-ND6 cells to undergo neuronal differentiation and regeneration processes in the presence of inhibitory cues, suggesting that NeuroD6 may alter the intrinsic properties of PC12 cells. To confirm these properties, we overexpressed NeuroD6 in mES cells using the retroviral vector MSinSB, which allows sustained transgene expression under the chicken actin promoter during mES cell differentiation. NeuroD6 was cloned in a bicistronic configuration with the enhanced green fluorescent protein reporter gene. Using an adherent monoculture differentiation protocol, we found that high NeuroD6 levels resulted in enhanced neuritogenesis and branching, as well as accelerated neuronal differentiation upon withdrawal of proliferative cues.

P-68 **Embryonic Stem Cells Genetically Engineered To Over-Express Gaba**

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Embryonic stem cell (ES) derived neurons represent a potential source of material for cellular transplantation into the brain. The ability to introduce transgenes into stem cells, to select and characterize clonal lines, and to direct those lines into neural phenotypes, permits opportunities to develop cell-based therapeutic strategies for intractable neurological diseases, such as epilepsy. Our previous studies have revealed a potential therapeutic effect of transplanted cells that over-express the inhibitory neurotransmitter γ–aminobutyric acid (GABA) in animal models of epilepsy. In this study we have genetically engineered the mouse ES cell line ZHTc6 to over-express the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD). The construct that was transfected permits regulation of the therapeutic transgene by tetracycline. Both isoforms of GABA synthesizing enzyme (GAD65 and/or GAD67) are being investigated. Two transfection methods, lipofection and electroporation, were evaluated for their yield of transfected ZHTc6 cells. Clonally selected cell lines were differentiated into predominantly neuronal phenotypes. The amount of GABA produced by differentiated and undifferentiated cell lines was measured *in vitro* using High-Performance Liquid Chromatography (HPLC). Transplanted cells were characterized for survival, migration and phenotype, using the reporter molecule □-glycosidase. We are currently investigating the potential for transplanted ES derived neurons

to suppress epileptic seizures. These studies should increase our understanding of the therapeutic potential of embryonic stem cells in neural repair.

P-69 **Barrier Reparative Potential Of Monocyte Cell Grafting In Spinal Cord Injury: An Em Study**

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Vascular injury and the related broken blood-cord barrier play a critical role in the irreversible pathophysiologic consequences in human spinal cord injury. The abnormal/defective vessel repair and the related compromised blood-spinal cord barrier lead to the secondary injury, the chronic inflammation and tissue degeneration and demyelination of the spared fiber tracts. Bone marrow-derived cells including cells of the monocyte/macrophage lineage have been shown to assume an endothelial-like phenotype in response to angiogenic factors, i.e., VEGF and play an important role in adult blood vessel growth and repair. It was demonstrated that VEGF pre-treated monocytes formed a vascular barrier *in vitro* and homed *in vivo* to injured blood vessels in an injured brain model [Glod *et al*, *Blood* (2006) **107**:940]. Here, the capacity of VEGF-treated monocytes to repair the injured blood vessels in injured rat spinal cord was examined by immuno-EM. In earlier studies it was demonstrated that the systemically administered VEGF-activated monocytes adopted endothelial-like characteristics and were found next to blood vessels, homing within the injured cord specifically at the lesion site in ratios >10:1. Further, these cells were associated with vessels that have an intact blood-cord barrier [Kalderon and Glod *Abstract Viewer SfN* (2005) Program No. 437.3. Online]. Here, a thoracic transection injury was performed in rat spinal cord and 7 days later $2x10^6$ VGEF-activated monocytes pre-labeled with micro-emerald dye (conjugated dextran-biotin-fluorescein) were grafted by *iv* injection. The fate of these monocytes was examined one week postgrafting by EM analysis for HRP-DAB labeling at the spinal cord lesion site. The grafted monocytes incorporated into the blood vessels and assumed endothelial position/structure, and a few of these showed also cell-junctions similar to tight junctions. It is suggested that systemic grafting of VEGF-activated monocytes could be developed into cell therapy for repairing the barrier following spinal cord injury. Supported by: NIH Grants, NS39375 (NK); NS4109, EY13145 and EY13079 (CA); and The New Jersey State Commission on Cancer Research 05-2002-CCR-E0 (JG).

P-70 **Banking Human Brain Tissue**

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Our Human Brain and Spinal Fluid Resource Center (Bank) has been devised to support neuroscience researchers. We collect and provide postmortem human nervous system specimens of high quality and sufficient quantity from specific neuropsychiatric diseases and disorders, as well as controls. It is an essential repository for scientists who utilize human postmortem or other tissues and body fluids (matched cerebrospinal fluid (CSF)/serum/plasma and urine) to test their hypotheses or apply their animal model finding to actual human disease.
The Bank serves as a bridge between clinicians and researchers. Clinicians furnish complete diagnoses (also biopsies) and CSF/blood specimens to the Bank. We bank pre- and postmortem specimens along with medical records. We coronally section and digitally image fresh brains, quick-freeze between 400-gram aluminum plates at –150°C. We save tissue in heat sealed bags and fluids in aliquots at -80°C. We validate primary clinical diagnoses with a neuropathological examination. We dissect regions of interest as requested by researchers. All dissections are horizontally bisected. One set of bisections is sent to the user and the other set is examined at the Bank for re-evaluation of pathology. MS dissections are 'triplets' (Plaque, NAWM & NAGM), which are similarly bisected. Upon request, type of plaque is classified. Lesions from other disease are also classified as needed by the users. Our quick frozen tissue has minimal ice artifact. We also collect matched pre- and post-mortem CSF and blood as well as frozen

CSF cells and blood leukocytes. We also have a collection of formalin fixed tissue. We help scientists' research studies that help development of diagnostic tests or markers of disease and may lead to new disease treatments. Please contact us to obtain neurospecimens free of charge for your research. Phone: (310) 268-3536, Fax: (310) 268-4768, email: brainbnk@ucla.edu, Web: www.loni.ucla.edu/uclabrainbank

P-71 **Neural Differentiation Potential Of Embryonic Versus Adult Stem Cells**

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In many central nervous system disorders such as spinal cord injury, multiple sclerosis, and amyotrophic lateral sclerosis loss of cells often leads to loss of function. Cell replacement therapies are a promising strategy to restore or repair these deficits. The amount and/or extent of the recovery may be associated with the purity of the stem cell-derived transplant population. Here, we investigated the differentiation potential of human embryonic stem cells (hESCs), human neural stem cells (hNSC), and human mesenchymal stem cells (hMSC). Our results show that hESCs and hNSC were capable of differentiating into high purity motor neuron progenitors displaying high levels of normal Tuj1 and Map2 staining, while hMSC retained their multipotent markers and displayed abnormal Tuj1 staining accompanied by extensive cell death following exposure to the differentiation paradigm. We also show that hESCs are capable of differentiating into high purity oligodendrocyte progenitors expressing high levels of GalC and O4, while hMSCs again retained their multipotent markers, displayed abnormal oligodendrocyte-lineage staining and were accompanied by extensive cell death following exposure to the differentiation paradigm. The hNSCs were capable of differentiating into mature oligodendrocytes, although in decreased numbers, as illustrated by GalC and O4 staining. Taken together, these data suggest that hESCs are the better source of stem cells with regards to efficiency and purity of neural differentiation products. In current studies, we are exposing mouse embryonic stem cell lines to our differentiation protocols, to determine their differentiation characteristics, and develop a useful means of generating high purity rodent cell populations for research.

P-72 **Optimization Of Human Embryonic Stem Cell-Derived Transplant Populations**

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Our lab and others perform transplants of defined cell populations into animal models of human injury and disease. We transplant human embryonic stem cell (hESC)-derived oligodendrocyte progenitor cells (OPC) and motor neuron progenitor cells (MNPC) into rodent models of spinal cord injury. The purpose of the work presented here is to determine the extent to which in vitro manipulation of cells decreases their viability. Preparation for transplant involves culturing and differentiating cells for weeks in advance. On transplant day, they are dissociated from their culture environment and stored on ice until they are ready for implantation. Assessment of success of these transplants includes quantification of transplanted cells surviving in vivo. An inability to distinguish between cell death due to hostility of transplant environment and cell death due to differential manipulation of cells before transplant is an unnecessary confound. hESC were cultured, differentiated into OPC or MNPC, and dissociated as though for transplant. The cells were then stored on ice and in increments of 15 minutes over a 3-hour period, plated onto an adherent substrate. The media was replaced 24 hours later and survival assessed

by staining with trypan blue and counting on a hemacytometer an additional 6 hours later. Lactate dehydrogenase (LDH) assays on the media showed the quantity that adherent and floating cells expressed of this marker of membrane degradation. MNPC viability initially remained high, but began dropping at 150 minutes, corresponding with an increase in LDH in the media. OPC viability steadily declined, corresponding with a steady increase in LDH in the media. These studies define the percentage of OPC and MNPC populations that are viable at time of transplant.

P-73 **Functional Characterization Of Human Embryonic Stem Cell Derivatives Following Lentiviral Transduction With Genes Containing Chondroitinase Or Adenylate Cyclase**

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Spinal cord injuries (SCI) are characterized by a loss of gray and white matter that affect the injury level as well as descending axons. Because of their capacity for virtually unlimited expansion and amenability for genetic manipulation, human embryonic stem cells (hESCs) offer great hopes for cell replacement strategies for SCI. Lentiviral vector technology has been shown to be highly effective to genetically engineer both undifferentiated and differentiated cells. We engineered a third-generation of self-inactivating (SIN) lentiviral vector with tetracycline inducible elements to control chondroitinase or adenylate cyclase transgene expression in a dose-dependent manner, in vivo and in vitro. We have used this vector to transduce hESCs, hESC-derived oligodendrocytes progenitor cells as well as hESC-derived motor neuron progenitor cells with each gene, and functionally characterized these cells using in vitro assays of axonal outgrowth.

P-74 **Human Embryonic Stem Cell Derived Motor Neuron Progenitor Cells Enhance Neuronal Survival And Neurite Outgrowth *In Vitro*: Implications For Spinal Cord Injury**

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Human embryonic stem cells (hESCs) are a valuable biological tool due to their ability to self-renew and develop into essentially any human cell type. Our lab and collaborators have devised protocols for the controlled differentiation of hESCs into high purity populations of oligodendrocyte and motor neuron progenitors (OPCs and MNPs, respectively). We have previously demonstrated that hESC-derived OPCs remyelinate axons and restore functional recovery following transplantation into an acute, thoracic contusion spinal cord injury (SCI) and secrete trophic factors that increase neuronal survival and process outgrowth both in vitro and vivo. Because OPCs and MNPs are derived from a common neural lineage, we investigated whether hESC-MNPs secrete growth factors that have trophic effects *in vitro* which could play a part in functional recovery following SCI. To address this, cortical neurons were exposed to either neural restrictive control media (NRM) or media conditioned by MNPs for 48 hours (MNP CM) and neurite outgrowth and neuronal survival were assessed. MNP CM enhanced neurite outgrowth of cortical neurons after 7 days in culture; an effect that was attenuated by antibody inhibition of candidate growth factors. As an acute inflammatory response and axonal severing accompany SCI, we sought to determine whether MNP CM has neuroprotective effects following these insults in vitro. MNP CM enhanced cortical neuron survival and viability in the presence of LPS-activated microglial CM. Following axotomy of cortical neurons in an isolated microfludic culture platform, MNP CM enhanced axonal regeneration nearly 50% more than NRM. These findings indicate that hESCs have the potential to not only replace cells lost to injury but to act as a vehicle for sustained neurotrophic release thus, protecting neural tissue following SCI.

P-75 **Human Embryonic Stem Cell-Derived Oligodendrocyte Progenitor Cell Transplants Improve Recovery After Cervical Spinal Cord Injury**

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Pre-clinical studies of spinal cord injury (SCI) primarily focus on thoracic level injuries, however most human injuries occur at the cervical level. Notable differences between the thoracic and cervical cord include afferent and efferent innervation, cord diameter, and proportion of gray to white matter. To assess cervical level SCI and the potential for cell-replacement therapies, we developed an animal model that uses the IH Impactor to deliver a defined bilateral spinal cord displacement, measured in kilodynes, at the C5 level in adult female Sprague-Dawley rats. The displacement produces reproducible histological deficits and concomitant forelimb behavioral profile, such as characteristic increases in pathological area of spinal cord and decrease in kinematic mean stride length, respectively. Importantly, injury parameters such as the affected volume, the amount of damage to both white and gray matter, and locomotion deficits in this cervical SCI model do not exactly correlate with these parameters in thoracic SCI. Therefore it is not obvious that treatments that improve functional outcomes in thoracic SCI will benefit cervical SCI. Our laboratory previously demonstrated that transplantation of human embryonic stem cell (hESC)-derived oligodendrocyte progenitor cells (OPCs) into adult rat thoracic SCI improved anatomical and behavior measures. Here, we report that transplantation of hESC-derived OPCs into acute (7 days) cervical SCI significantly improved forelimb locomotion outcomes that correlated with improved histological measures. In addition, cells localized to the injury site and did not form gross tumors. These results provide further evidence of the therapeutic potential and broaden the application of hESC-derived OPCs for the treatment of SCI.

P-76 **Propriospinal Neuron Plasticity After Spinal Cord Transection Is Associated With The Development Of Autonomic Dysreflexia**.

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Spinal cord injuries above the sixth thoracic (T6) level frequently result in the development of autonomic dysreflexia, an abnormal hypertensive condition commonly triggered by painful sensations below the injury level. We have shown that post-traumatic nerve growth factor (NGF)-induced sprouting of nociceptive fibers into the lumbosacral spinal cord is correlated with elevated hypertension in response to colorectal distension (CRD). We are now studying neural substrates which relay noxious visceral input to activate disinhibited rostral sympathetic neurons after injury. The anterograde tract tracer biotinylated dextran amine (BDA) was injected unilaterally into the L6/S1 dorsal commissural nucleus (DCN) in T4-transected and sham rats. After 2 weeks, densitometric analysis showed significantly more BDA^+ fibers in both ipsi- ($p<0.01$) and contralateral ($p<0.05$) thoracic gray matter of injured cords versus shams. To corroborate these findings and retrogradely label putative lumbosacral propriospinal neurons, fast blue or cholera toxin beta was injected bilaterally into the T9 dorsal horns 2 weeks post-injury versus shams. Stereology demonstrated significantly ($p<0.05$) more labelled DCN neurons (T13-S1) in injured cords versus shams one week later. Additionally, following CRD the number of c-Fos^+ cells significantly ($p<0.01$) increased in the DCN of injured versus sham cords. This infers that increased activity and plasticity of lumbosacral propriospinal neurons convey noxious visceral input rostrally after injury to elicit autonomic dysreflexia. Currently, we are using pseudorabies virus (PRV) expressing either GFP or RFP injected into the kidney and distal colon to transynaptically label sympathetic preganglionic and lumbosacral propriospinal neurons, respectively. Axonal remodelling after injury (GFP/RFP co-localization) is being quantified to assess the convergence of distinct renal and colonic neuronal pathways. We are also employing patch-clamp recording techniques in acute slice preparations from T4-transected cords versus shams to compare baseline neuronal activity of PRV-labelled lumbosacral

propriospinal neurons, and in response to afferent stimulation (i.e. application of glutamatergic agonists). *Supported by KSCHIRT #3-11 and NIH/NINDS R01 NS049901-01-AGR.*

P-77 **Acute Transplantation Of Olfactory Ensheathing Cells Promotes Recovery From Autonomic Dysreflexia In T4 Spinal Cord Transection**

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Aim: Autonomic dysreflexia is an episodic disturbance of cardiovascular functions in subjects with spinal cord injury above the level of T6. In this study we examined the effect of transplanted olfactory ensheathing cells on cardiovascular responses in rats with spinal cord injury. **Methods:** Animals were implanted with a radio-telemetric transmitter for blood pressure monitoring. T4 transection was followed by transplantation of biodegradable gelatine sponge soaked with olfactory ensheathing cells (cell-treated, $n=10^6$) or culture medium (control group) inserted between the cut surfaces. We used colorectal distension to induce autonomic dysreflexia expressed as an increase in blood pressure and reflex drop of heart rate. **Results:** Autonomic dysreflexia was fully developed 3 weeks after the spinal cord transection. The cellular therapy did not significantly effect the maximum changes in blood pressure and heart rate, however the trend towards decreased peak blood pressure was observed in cell-treated group ($p=0.086$). The recovery of blood pressure in cell-treated rats was faster with T50 (time for the blood pressure to recover to 50% of the maximum deviation elicited by colorectal distension) and significantly shorter (cell-treated: 159.7±3.2 s; control: 183.8±22.9 s mean±SD; $p<0.05$). We have not observed any difference in baseline heart rate and blood pressure between the groups. **Conclusion:** Transplantation of olfactory ensheathing cells at the site of a transection spinal cord injury assists in limiting the duration of the hypertension associated with autonomic dysreflexia. This could result from trophic/regenerative changes in the spinal cord above or below T4. Further histological analysis is necessary to examine structural effects of the transplanted cells on the host spinal cord.

P-78 **Neuroplasticity In The Respiratory Circuit Following Lateral Hemisection In The Adult Rat Cervical Spinal Cord**

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Following a lateral, high cervical spinal cord injury, phrenic motoneurons (PhMNs) exhibit spontaneous recovery of neurophysiological activity despite loss of ipsilateral inspiratory drive. Therapeutic approaches for enhancing respiratory neuroplasticity and recovery following such injury are currently being investigated. However, further definition of the neural circuitry associated with phrenic motoneuron (PhMN) function is an essential first step. Adult female Sprague-Dawley rats were divided into two groups – unoperated controls and injured (left-sided, C_2 hemisection) animals – for trans-synaptic tracing using pseudorabies virus (PRV). Animals were anesthetized, the diaphragm surgically exposed, and PRV was administered to the left half of the diaphragm. Animals were collected 48-72hr post-PRV (pPRV) delivery. Only ipsilateral PhMNs were infected >48hrs pPRV; medullary neurons were not infected until 72hrs. At 60-64 hrs pPRV, a population of infected neurons was seen both ipsi- and contralateral to the labeled hemidiaphragm, distributed primarily in the region of Rexed laminae VII and X. A similar labeling pattern was seen 1-4 wks following C_2 hemisection. As these neurons were PRV infected after PhMNs, but prior to supraspinal labeling, we conclude these spinal interneurons are premotor to PhMNs. Labeling left and right halves of the diaphragm with PRV expressing green

(PRV152) and red fluorescent (PRV614) protein respectively, demonstrated double-labeling in some of these cells. Immunostaining with antibodies to choline acetyl-transferase (ChAT) demonstrated that some of these interneurons were cholinergic. These findings demonstrate the presence of a bilaterally distributed interneuron population in the region of the phrenic nucleus (C_3-C_5), some of which project to both left and right PhMNs. Ongoing studies involving anterograde labeling of physiologically-identified rVRG neurons have also demonstrated collateral projections to cervical interneurons. The functional significance of these cells normally and during partial recovery of ipsilateral PhMN function in this lesion model awaits further investigation.

P-79 **A Diketopiperazein Dkp101516 Stimulates The Sprouting Of Spinaly Projecting Axons**

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Following spinal cord injury, reinnervation is hindered by the expression of a myriad of inhibitory molecules in the adult central nervous system. In both the astroglial scar and throughout the spinal cord, chondroitin sulfate proteoglycans (CSPGs) and myelin associated proteins (MAPs) work in concert to inhibit axon regeneration and compensatory sprouting through their collapsing effects on the neuronal growth cone. The neuronal growth cone is a motile structure at the tips of growing axons and at sites along the axon where branches are initiated. Inhibitors such as MAPs and CSPGs cause the growth cone collapse and therefore hinder axon outgrowth and branching. To overcome the effects of these inhibitors, we focused on identifying drugs that can stimulate the motility of neuronal growth cone. The neuronal growth cone has the characteristic structure of motile cells, where the movement of the growth cone is regulated by a similar family of Rho GTPases as in motile cells. In this study, we have identified a compound that stimulates cell motility: a diketopiperazine designated DKP101516. Our studies demonstrated for the first time that a compound stimulating cell motility DKP101516 can enhance axon outgrowth and branching in cortical neurons *in vitro*. Further *in vivo* analysis demonstrated that DKP101516 enhances the plasticity of various axonal populations following septuple dorsal rhizotomy by overcoming the inhibitory effects of CSPG and MAPs. In particular, DKP101516 enhances the sprouting of injured primary afferents and monoaminergic axons. However, DKP101516 appears to exacerbate astrogliosis and neurocan expression in an injury dependent manner, and therefore hinders the regeneration of injured primary afferents across the DREZ. Collectively, our results suggest that although DKP101516 fails to promote axon regeneration, it may encourage spinal cord repair by stimulating compensatory sprouting of intact axonal projections. Funding: Rick Hansen Man in Motion, CIHR.

P-80 **Hud-Binding Motif In Gap-43 Mrna 3'utr Directs Its Subcellular Localization In Adult Rat Drg Neurons**

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The growth associated protein GAP-43 plays essential roles in mammalian PNS/CNS axonal growth, both during development and regeneration. In recent years it has become clear that the 3' untranslated region (UTR) of GAP-43 mRNA contributes to its stability and translation through binding to the ELAV-like protein, HuD. GAP-43 mRNA and HuD have been shown to localize to neuronal processes. Here, we have asked whether the 3'UTR of GAP-43 mRNA also regulates its localization into axons. For visualizing the subcellular localization and translation of GAP-43 mRNA, we generated destabilized GFP^{myr} reporters containing the full length, 5' proximal, or 3' distal segments of GAP-43

mRNA's 3'UTR (*dzGFP-$^{898\text{-}1483}$GAP43, dzGFP-$^{898\text{-}1053}$GAP43, and dzGFP-$^{1067\text{-}1281}$GAP43*, respectively). These constructs were transfected into adult DRG cultures and live cell imaging and immunocytochemistry were employed to visualize localized translation products of the axonally localized reporter mRNAs. Only GFP reporters containing the full or distal segment of the 3'UTR showed focal expression of GFP signal in distal axons. The *dzGFP-$^{898\text{-}1053}$GAP43* reporter showed weak homogenous signals along the axons. Both the *dzGFP-$^{898\text{-}1483}$GAP43* and *dzGFP-$^{1067\text{-}1281}$GAP43* reporters showed fluorescence recovery in the distal axons over 8-10 min after photobleaching (*i.e.*, FRAP). Moreover, this recovery was completely blocked by pretreatment with the translation inhibitor anisomycin consistent with localized translation of the reporters in distal axons. The *dzGFP-$^{898\text{-}1053}$GAP43* showed no significant recovery after bleaching even when translation was intact, indicating that its axonal signals are due to diffusion of reporter synthesized in the soma. Together, these data indicate that the 3'UTR sequences contained within residues 1067-1281 are necessary and sufficient for transport of GAP-43 mRNA into regenerating sensory axons. This region of GAP-43 contains the HuD binding site. Thus, these studies suggest that HuD binding to GAP-43 mRNA 3'UTR can not only increase the level of GAP-43 mRNA via stabilization, but also direct its subcellular localization. [*These studies were supported by funds from NIH, the Dr. Miriam and Sheldon Adelson Medical Research Foundation, and the Nemours Foundation*]

P-81 **Dependence Receptors And Retrograde Neuronal Death After Spinal Cord Injury**

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Lampreys recover from complete spinal transection and axons regenerate selectively in their correct paths. However, only about 50% of the severed reticulospinal axons regenerate through the site of injury, while the fates of unregenerated neurons remain unknown. We now report that in animals allowed to survive from 4 to16 weeks after spinal cord transection, several identified reticulospinal neurons were missing in Nissl-stained brain. TUNEL - positive neurons were detected at 2 - 16 weeks post-transection, suggesting that some cells were dying by apoptosis. The majority of these neurons also expressed UNC-5 or Neogenin. Of special interest, expression of netrin and RGM, ligands for UNC-5 and Neogenin respectively, were greatly downregulated after spinal cord transection. We hypothesize that expression of UNC-5 or Neogenin in these neurons after spinal cord injury combined with downregulation of netrin and RGM near the lesion may lead to neuronal death. UNC-5 and Neogenin are known to be dependence receptors, *i.e.*, they can induce apoptosis when unoccupied by their ligands, netrin or RGM, but inhibit apoptosis in the presence of ligand. Neurotrophins reduce neuronal atrophy and promote regeneration following spinal cord injury. We assessed expression of neurotrophin (NT) and it Trk receptor by *in situ* hybridization in control animals and after spinal cord transection. NT expression was downregulated in neurons close to the transection starting 2 weeks post-transection but upregulated in microglial cells caudal and rostral to the lesion. By 4 months after transection, NT expression was not detected in the spinal cord close to transection site. Trk expression was not change 2 and 4 weeks after spinal cord injury but was completely absent 4 months after transection. We hypothesize that the balance between dependence receptors and neurotrophin receptor determines neuronal survival level. Supported by NIH grants R01 NS38537 and R24 HD050838.

P-82 **Cytokine And Tissue Responses To Acute Interferon-Beta Administration After Cervical Contusion Spinal Cord Injury**

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Secondary degeneration produces an expansion of the initial tissue damage sustained by a spinal cord injury (SCI). Dampening the cellular inflammatory response that contributes to the delayed tissue

damage is one strategy for neuroprotection after SCI. In this study we examined whether treatment with a PEGylated form of rat interferon-beta (IFN-β) improved forelimb function and/or promoted structural and molecular changes at the lesion site following a unilateral cervical level contusion. SCI was administered to adult female Sprague-Dawley rats with the Infinite Horizon Impactor at a force of 200Kdyne (equivalent to a severe injury) and a mean displacement of 1600-1800 □m. A single (5×10^6 units) dose of PEGylated IFN-β or vehicle was administered 30 minutes following SCI. Weekly behavioral tests to assess locomotion (forelimb locomotor scale (FLS), hindlimb BBB and TreadScan) along with sensorimotor function (grid walking and grooming) were carried out. Evaluation of survival of brainstem neurons, lesion size, presence of phagocytic cells, and glial cell reactivity will be reported. Preliminary data with an acute low dose (5×10^4 units) treatment showed a trend towards a decrease in the pro-inflammatory cytokines IL-1□, IL-1□ and IL-6 and an increase in the anti-inflammatory IL-10 within the lesion epicenter at 12 hours post SCI. Cytokine levels from other groups sacrificed at 6, 24, 48, or 72 hours after treatment with the higher dose (5×10^6 unit) of PEGylated IFN-β will be reported as an indication of possible neuroprotective results from this treatment strategy. Supported by NIH/NINDS NS26380.

P-83 **Evidence That A Critical Number Of Regenerating Axons Is Necessary To Form A Topographic Retinotectal Projection**

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The goldfish retinotectal system is a well-characterized model of successful CNS regeneration. When the optic nerve of an adult goldfish is severed, retinal ganglion cell (RGC) axons regenerate to their primary target, the optic tectum, to reestablished precise topographic connections. In mammals, strategies to promote CNS axon regeneration have met with some success, but typically only a small number reach their targets. We asked how well goldfish RGC axons would reform a topographic projection if only a fraction of them invaded tectum.

To generate a “low-density” optic projection, we completely denervated one tectum by removing its contralateral eye and crushed the optic nerve of the remaining eye. While most axons regenerated into the correct contralateral tectum, retrograde labeling with Fluorogold revealed that approximately 10-20% of axons regenerated into the incorrect ipsilateral tectum. After 9-12 months, nanoliter spot injections of DiI were made into retina to label a small number of neighboring RGCs and the distribution of their axons was examined in tectal flat mounts. Little topographic order was observed. Although most (but not all) axons were in the correct mediolateral half of tectum, these were dispersed over a large area. Only a few of these axons were able to converge on their estimated target zone; about 70% of axons were outside of their topographic area. This contrasts to the normal density regenerated projection (nerve crush with regeneration into contralateral tectum) in which only 5% of axons were outside of their target zone and none were seen in the incorrect half of tectum. In the low density projection, axonal morphology was also highly abnormal. Instead of forming a single terminal arbor, ectopic branching was frequently seen protruding from the axonal shaft. These were never observed in regenerated fibers in a normal density projection. Supported by NIH grant EY014200 to RLM.

P-84 **Visual Restoration And Transplant Connectivity In Degenerate Rats Implanted With Retinal Progenitor Sheets**

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Purpose: To study interactions between retinal progenitor layer transplants and degenerating host retina. **Methods:** Donor retinal sheets, isolated from E19 rat fetuses expressing human alkaline phosphatase (hPAP), were transplanted to the subretinal space of 3.5-5 week old S334ter-3 rats with fast retinal degeneration. Recipients were sacrificed at the age of 7-43 weeks. Visual responses were recorded electrophysiologically in the superior colliculus (SC) in selected rats at the age of 23 to 32 weeks. Vibratome slices were stained for the donor marker hPAP, and processed for EM. **Results:** All recorded transplanted rats had restored or preserved visual responses in the SC corresponding to the transplant placement in the retina, with thresholds between -2.8 and -3.4 log cd/m^2. No such responses were found in age-matched S334ter-3 rats without transplant. Donor cells and processes could be identified in the host by light and electron microscopy. The hPAP antibody penetrated approximately 10 µm of the vibratome slice surface. Transplant processes penetrated the inner host retina in spite of occasional glial barriers between transplant and host. Labeled neuronal processes were found in the host inner plexiform layer, sometimes forming synapses with unlabeled presumably host cells. **Conclusions:** Results indicate that synaptic connections between graft and host contribute to visual responses in the brain, supporting previous results of restored visual function and synaptic connectivity. **Support:** Foundation Fighting Blindness, Lincy Foundation. MJS and RBA have proprietary interests in the implantation instrument and procedure.

P-85 **Csf Biomarkers Of Injury Severity After Spinal Cord Injury**

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Currently, the severity of human spinal cord injury (SCI) is classified according to the extent of motor and sensory loss. On a practical level, such functional measures are often impossible to ascertain in some patients (eg. with head injury, intubation/sedation). On an academic level, such functional measures are used in clinical trials to provide only a gross stratification of neurologic impairment (ASIA A, B, C, and D), making it necessary to enrol large numbers of patients to sufficiently power clinical trials. A biomarker of injury severity that more precisely represented the biological extent of spinal cord damage would therefore be invaluable both clinically for determining injury severity and scientifically for more accurately stratifying patients into clinical trials of novel therapies. We have attempted to establish such biomarkers by biochemically evaluating the cerebrospinal fluid (CSF) of human patients after SCI. 20 patients enroled in a prospective clinical trial had samples of CSF withdrawn through an intrathecal drain for 72 hours. The cohort included 13 males, 7 females, with an average age of 41.9 years. Neurologic impairment was classified as ASIA A (n=13), ASIA B (n=4), and ASIA C (n=3). Multiplex and standard ELISA analyses revealed a severity-dependent expression of a number of inflammatory cytokines. Ordinal logistic regression was performed on the concentrations of these proteins at the 24 hour time point, and prediction models using various combinations were generated. The actual concentrations were then fed into the model to evaluate how well they predicted the observed severity of injury. Using a combination of proteins, this generated a model with an 84.2% accuracy rate of predicting the observed ASIA impairment grade (A, B, or C). These findings represent a first attempt at utilizing a biochemical evaluation of human cerebrospinal fluid to establish a biological surrogate of neurologic impairment after spinal cord injury.

P-86 **The Effects Of Combined Lesions To Dorsolateral And Dorsal Funiculus In Rats**

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The purpose of this research was to investigate the compensatory role of undamaged spinal pathways after partial spinal injury in rats. We have previously shown that bilateral lesions of the dorsolateral funiculus (DLF) at the cervical level caused minor but persistent changes in overground locomotion and also caused moderate deficits during skilled limb use, particularly in the hindlimbs. We hypothesized that the corticospinal tracts (CST), located within the undamaged dorsal funiculus (DF), might be compensating for loss of DLF input to the forelimbs. To test this, we performed bilateral DF lesions in animals in which both DLFs had been transected 6 weeks previously. These secondary DF lesions involved either only ascending sensory pathways (ASP group) in the DF, while sparing the CST or involved both the ASP and the CST (ASP+CST group). All animals were assessed during overground locomotion, while crossing a horizontal ladder and during a pellet reaching task. During overground locomotion, both groups moved with slightly altered forces and timing in both fore and hindlimbs. In contrast, both groups made many more errors with both fore- and hindlimbs during ladder crossing compared to either DF or DLF lesions alone. During reaching, ASP lesions in DLF rats caused more severe deficits compared to DLF lesions or ASP lesions alone, whereas ASP+CST lesions in DLF rats resulted in deficits which were similar to ASP+CST lesions alone. Thus, it appears that DF pathways, particularly the ASP, do provide some compensatory input in animals after DLF lesions, but these are confined to skilled movements such as ladder locomotion and forelimb pellet retrieval. In other behaviours, such as overground locomotion, the effects of both lesions appear to be additive. Importantly, these compensatory changes need time to develop, because animals with ASP+CST+DLF lesions performed simultaneously display marked functional deficits and are unable to use their forelimbs for locomotion or reaching.

P-87 **Peri-Lesion Growth Factor Delivery Prevents Corticospinal Atrophy Following Non-Human Primate Spinal Cord Injury**

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Spinal cord injury disconnects cortical neurons from their spinal targets. Whether these corticospinal neurons die or become atrophic is controversial. To examine the cell body response to primate spinal cord injury, ten adult rhesus monkeys underwent unilateral transections of the C5/C6 cervical segment. Five subjects were treated with autologous fibroblast grafts secreting BDNF and NT-3 into the lesion site, combined with injections of lentiviruses expressing NT-3, rostral and BDNF, caudal to the lesion. Six months later, subjects were sacrificed, and cortical sections were stained with cresyl violet. Stereological counts of layer 5 neurons within the contralateral primary motor cortex revealed no significant differences in total numbers between groups: intact, 261,477$\pm$ 20,861; lesioned, 275,486 $\pm$ 16,097; treated, 247,681 $\pm$ 19,995 (ANOVA p-value = 0.74). However, analysis of the size distribution of layer 5 neurons revealed significant differences between the groups in the percentage of large neurons (area >500 um^2). Compared to the intact group, the lesion-only group exhibited a 75.5 $\pm$ 1.2% decrease in large neurons ($p<0.001$ compared to intact), while the treated group exhibited significant protection from lesion-induced atrophy, with a loss of only 27.4 $\pm$ 3.5% large neurons ($p<0.05$ compared to intact, $p<0.001$ compared to lesioned). These results suggest that following non-human primate spinal cord injury: 1) corticospinal neurons become atrophic but do not exhibit significant overall cell death, and 2) growth factor delivery within the site of injury, a substantial distance from the cortex, can prevent lesion-induced neuronal atrophy. Thus, injured corticospinal neurons of the non-human primate retain the ability to respond to trophic signals provided within the axotomized region. These findings have important implications for maintaining an injured neuron in a state more supportive of plasticity and regeneration, on a scale relevant to human injury.
Supported by the VA, NIH and the California Roman Reed Research Bill.

P-88 **Presence Of Serotonergic And Noradrenergic Axons In The Caudal Stump Following Complete Spinal Cord Transection Is Not Exclusive Evidence Of Regeneration**

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Two widely used immunocytochemical markers of axon regeneration following complete spinal cord transection are serotonin (5-HT) and dopamine beta-hydroxylase (DBH), which often are presumed to identify the raphespinal and coeruleospinal pathways, respectively. As these descending pathways modulate locomotion, we examined their contribution to the functional recovery reported in completely transected, OEG-injected adult rats (Kubasak et al., 2005). We detected 5-HT-positive (5-HT+) axons crossing the GFAP-negative transection site in OEG-injected rats, findings indicative of raphespinal regeneration. However, we found substantial numbers of 5-HT+ fibers and occasional 5-HT+ interneurons within lamina X at lower thoracic, lumbar and sacral levels of media- and OEG-injected rats. Interestingly, 5-HT+ axons in the caudal stump appose two populations of locomotion-associated cholinergic neurons, central canal cluster cells and somatic motor neurons, with more fiber appositions detected in OEG- than media-injected rats.

Although DBH+ axons crossed the GFAP-negative scar and entered the caudal stump in both media- and OEG-treated spinal rats, the density of DBH+ fibers in the segment just caudal to the transection site was greater in OEG- than media-injected rats, findings consistent with coeruleospinal regeneration. We then analyzed the density of DBH+ axons at lower thoracic, lumbar and sacral segments and found sparse but equal densities of DBH+ axons in all animals. DBH+ fibers often extended along the pia-arachnoid and occasionally within the ventral and dorsal roots. Double labeling experiments revealed DBH+ axons coursing between the meninges and the spinal cord along large penetrating blood vessels, suggesting that these axons may be of peripheral origin. Regardless of their source, 5-HT+ and DBH+ axons in the caudal stump may contribute to the functional recovery following spinal cord transection. Neither the presence of 5-HT+ nor DBH+ axons in the caudal stump, however, appears to be a reliable indicator of raphespinal or coeruleospinal regeneration. Supported by: NIH RO1NS54159.

P-89 **Compared Histological, Morphometrical And Functional Evaluation Of Axonal Regeneration Of Transected Adult Rat Peripheral Nerves After End-To-End And End-To-Side-Anastomsis**

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The gold standard for reconstruction of transected peripheral nerves is tension-free end-to-end coaptation of the nerve stumps. End-to-side neurorrhaphy is a surgical intervention for peripheral nerve reconstruction where no proximal nerve end is available or end-to-end neurorrhaphy not feasible. Although axonal and functional regeneration has been confirmed experimentally and clinical reports show encouraging results, the cellular mechanism behind this remained unclear. Therefore, we performed tibial nerve end-to-end in comparison to tibial-to-peroneal nerve end-to-side anastomosis through a perineurial window in adult rats. Six weeks after surgery the nerves were dissected and electrically stimulated to evaluate functional recovery by means of electromyography from the gastrocnemius muscle. Retrograde tracing of neurons projecting into both nerves was performed in four out of eight animals per group by placing crystals of DiI (tibial nerve) or FluoroGold (peroneal nerve) at the transected stump 10 days prior to sacrifice of animals. The coaptation site was paraffin embedded and immunocytochemical analysis verified regenerated axons in both groups. Morphometrical quantification of all myelinated axons was done in semithin sections from epon-embedded tissue proximal and distal to the coaptation sites and was compared to healthy non-operated nerve samples. No significant difference between both reconstruction methods could be drawn from electromyography. An increase in retrogradely double-labelled neurons projecting into the nerves coapted by end-to-side

neurorrhaphy as compared to healthy controls, indicates collateral sprouting of donor axons into the recipient nerve. Morphological results demonstrate elevated numbers of myelinated axons in the donor nerve proximal to the end-to-side anastomosis compared to control tissue. However, significantly higher numbers of regenerated myelinated axons are found distal to end-to-end as compared to end-to-side repair. Furthermore, regarding quantitative parameters such as axon diameter, axon area, fibre density and g-ratio-index, impairment of the donor nerve is not evident. Thus, end-to-side neurorrhaphy represents a valuable alternative to end-to-end neurorrhaphy.

P-90 **Novel Role For Aquaporin-1 In Axonal Growth**

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The role of water channel aquaporin 1 (AQP-1) in spinal cords is unknown. AQP-1 is one of several water channels that mediate water and ion movement in the brain and it has been shown to regulate production of cerebrospinal fluid (CSF). Here we propose that the primary role of AQP-1 in spinal cords is the water transport associated with axonal growth, in both uninjured and injured spinal cords. We found that moderate rat contusion SCI induces persistent and significant 4-8 fold increases in AQP-1 protein levels, not only at the site of injury (T10), but also throughout injured spinal cords, which lasts up to 11 months post-contusion. AQP-1 is - similar to axonal growth-associated protein 43 (GAP43) - most robustly expressed in small diameter sensory fibers of the dorsal horn in uninjured spinal cords. After SCI, however, AQP-1 expression increases in all surviving spinal neurons and axons, including dorsal horn sensory fibers. We also found that oxidative or osmotic stress after SCI significantly and persistently increases synthesis of AQP-1 and GAP43 (e.g. axonal growth), because anti-oxidant melatonin or isotonic solution both decrease AQP-1 and GAP43 expression in injured spinal cords. Therefore, it appears that AQP-1 may have a novel and important role in axonal reorganization and plasticity selectively occurring in small diameter sensory fibers in dorsal horns of adult uninjured spinal cords. However, significantly elevated AQP-1 levels in almost all surviving neurons/axons in injured spinal cords may contribute to non-specific axonal sprouting and impaired axonal regeneration after SCI.

P-91 **Fibrin-Based Tissue Engineering Scaffolds For The Treatment Of Subacute Spinal Cord Injury**

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The purpose of this study was to evaluate the effects of fibrin based scaffolds on regeneration following subacute rat spinal cord injury (SCI). In previous *in vitro* studies, fibrin scaffolds modified with a heparin-binding delivery system (HBDS) were optimized for the controlled release of neurotrophin-3 (NT-3). Based on these results, fibrin scaffolds were evaluated for their morphological effect on a subacute spinal cord injury model (treatment 2 week after injury). For the first part of this study, 6 Long Evans rats were anesthetized and injured with a dorsal hemisection SCI. Two weeks following initial injury, the lesion site was re-exposed, scar tissue was removed, and a fibrin scaffold was implanted into the wound site. At 2 and 4 weeks following implantation, the treated spinal cords were harvested and evaluated for morphological differences using markers for neurons, astrocytes, and chondroitin sulfate proteoglycans. Compared to controls, rats treated with fibrin scaffolds had significantly higher neural fiber (beta-tubulin III) staining in the lesion site and significantly less astrocyte (GFAP) staining at the lesion border. Next, the effect of controlled release of NT-3 from fibrin scaffolds was evaluated for a series of doses (500, 1000, and 2000 ng/mL). At 2 weeks following implantation the treated spinal cords were harvested and evaluated for morphological differences using the same markers. These results suggest that fibrin scaffolds have potential as treatment for SCI and future studies will evaluate the effect of embryonic stem cell derived neural precursors embedded in the scaffold.